Cognitive, behavioural, environmental and genetic associations of myopia

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Cognitive, behavioural, environmental and genetic associations of myopia

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Declaration

I, Katie Williams, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Katie M Williams

Date 26th February 2017

Abstract

This thesis, presented as a thesis incorporating publications, examines the epidemiology, aetiology and genetics of myopia. It comprises work utilising two twin cohorts, Twins Early Development Study (TEDS) and TwinsUK, and collaborative work with the European Eye Epidemiology consortium (E^3), EUREYE Study and Consortium for Refractive Error and Myopia (CREAM).

The prevalence of refractive error across Europe was defined using a large, meta-analysis (E^3). Myopia prevalence was higher in younger generations (47% in 25-29 year-olds), with clear evidence of higher rates for those born in the latter half of the twentieth century. There was a strong association between myopia and education – in E^3 myopia prevalence was almost double in those with higher compared to primary education (43% vs. 24% in 50-54 year-olds). Similarly in EUREYE those going onto higher education had twice the odds of myopia (OR 2.08). In TEDS educational attainment and cognitive ability were associated with myopia only in adolescence; significant factors maintained in life-course models were maternal education (OR 1.33), fertility treatment (OR 0.63), summer birth (OR 1.93), and video games (OR 1.03). Time outdoors was replicated to be inversely associated with myopia in EUREYE, particularly in younger life (OR 0.81), but no evidence for mediation by vitamin D was observed.

The complex interplay between environmental and genetic factors was examined in TEDS. Evidence of a shared genetic risk for myopia and higher intelligence was demonstrated using twin modeling (78% of phenotypic correlation explained by genetic factors) and polygenic risk scores. A suggestive interaction between genetic variants for myopia and near work was identified for five loci in CREAM.

Myopia is becoming more common in Europe. Replicated and novel associations with myopia reflect societal trends and the complex, powerful interplay between genetics and environment. This has implications for future risk prediction and therapeutic interventions.

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Table of Contents

Declaration 2

Abstract 3

Acknowledgments 4

Publications arising from this work 8

List of Figures 12

List of Tables 15

Abbreviations 17

Chapter 1 | Introduction 19

1.1 Overview 20

1.2 Definition and Clinical Aspects of Myopia 20

1.21 What is myopia? 20

1.22 Definitions of myopia and natural history 21

1.23 Refractive correction of myopia 25

1.24 Pathological complications of myopia 26

1.3 Epidemiology of Myopia 29

1.31 Epidemiology of myopia in adults 29

1.32 Epidemiology of myopia in children 32

1.33 Projections and implications 36

1.4 Environmental factors for Myopia 36

1.41 Education 37

1.42 Intelligence 41

1.43 Near work 42

1.44 Time outdoors 45

1.45 Urbanisation 49

1.46 Socioeconomic Status 50

1.47 Parental factors 50

1.48 Others 51

1.5 Genetic aspects of myopia 52

1.51 Twin Studies 52

1.52 Genome-wide association studies 56

Chapter 2 | Aims of Thesis 67

Chapter 3 | The changing epidemiology of myopia in Europe and the United Kingdom 70

3.1 Introduction 71

3.2 Prevalence of refractive error in Europe 72

3.3 Increasing prevalence of myopia in Europe and the impact of education 85

3.4 Age of myopia onset in a British population-based twin cohort 96

Chapter 4 | Environmental factors for myopia 107

4. 1 Introduction 108

4.2 Early life factors for myopia in the Twins Early Development Study 109

4.3 The association between myopia, ultraviolet B exposure, and vitamin D 138

Chapter 5 | Genetic factors for myopia and the interplay with environmental risk factors 150

5.1 Introduction 151

5.2 Heritability and associated genetic variants for refractive error in TEDS 151

5. 21 Classical twin study in TEDS 151

5.22 Genome-wide Association Study in TEDS 155

5.3 The use of proxies in GWAS studies of refractive error 160

5.4 Phenotypic and genotypic correlation between myopia and intelligence 164

5.5 Childhood gene-environment interactions 173

Chapter 6 | Discussion and Conclusions 204

6.1 Introduction 205

6.2 Epidemiology of myopia 205

6.3 Environmental aetiology of myopia 208

6.4 Genetic aetiology of myopia 210

6.5 Contributions to the literature and implications 212

6.6 Limitations 214

6.7 Future directions 217

6.8 Conclusions 222

Chapter 7 | Materials 224

7.1 Introduction 225

7.2 The Twins Early Development Study 225

7.3 Data Collection 226

7.4 Refractive error classification 227

7.5 Assessment of representativeness of cohort **228**
7.6 Distribution and prevalence of refractive error **229**
References **234**
Appendix **254**
8.1 TEDS Myopia Study - questionnaire to the twins **255**
8.2 TEDS Myopia Study - questionnaires to optometrists **256**
8.3 TEDS Myopia Study Ethical Approval **260**
8.4 Supplementary Information for cohorts contributing to the presented E³ analyses **264**
8.5 Supplementary Information for the cohorts contributing to the presented Consortium for Refractive Error and Myopia (CREAM) analysis **271**

Publications arising from this work

Williams KM, Bentham GCG, Young IS, McGinty A, McKay GJ, Hogg R, Hammond CJ, Chakravarthy U, Rahu M, Seland J, Soubrane G, Tomazzoli L, Topouzis F & Fletcher AE. Association Between Myopia, Ultraviolet B Radiation Exposure, Serum Vitamin D Concentrations, and Genetic Polymorphisms in Vitamin D Metabolic Pathways in a Multicountry European Study. JAMA Ophthalmology. 2017 Jan 1;135(1):47-5

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List of Figures

Chapter 1 Introduction

Figure 1.1 Emmetropia, axial myopia, refractive myopia secondary to corneal curvature and refractive myopia secondary to cataract 21
Figure 1.2 Concave lenses are used to diverge incipient rays of light in a myopic eye to ensure light is focussed onto the retinal plane 22
Figure 1.3 Odds ratios for retinal detachment associated with different grades of myopia 26
Figure 1.4 Features of pathological myopia 27
Figure 1.5 Forest plot of odds ratios for myopic maculopathy with increasing myopia 29
Figure 1.6 Changes in the prevalence of myopia in the three major ethnic groups in Singapore 30
Figure 1.7 Prevalence of myopia in school children in Taiwan across five examination periods 33
Figure 1.8 Extracts from Edward Nettleship's 1879 'Student's Guide to the Diseases of the Eye' 43
Figure 1.9 Multivariable-adjusted odds ratios for myopia by reported average daily hours spent on near work versus outdoor activities in 12 year-olds 47
Figure 1.10 Peripheral hyperopic defocus 48
Chapter 1.52 | Figure 1 Heritability estimates for refractive error 58
Chapter 1.52 | Figure 2 Studies, traits and SNP-trait associations from 2005 to 2013 reveal the growth in genome-wide association studies 59
Chapter 1.52 | Figure 3 Manhattan plot of genetic associations for refractive error in the CREAM combined GWAS meta-analysis 60
Chapter 1.52 | Figure 4 Network connections of genes associated with myopia 61
Chapter 1.52 | Figure 5 Minor allele frequency against effect size for the significant variants identified in the CREAM GWAS 62

Chapter 3 The changing epidemiology of myopia in Europe & the UK

Chapter 3.2 | Figure 1 Flow chart of refractive error meta-analysis in E^3 78
Chapter 3.2 | Figure 2 Distribution of refractive error 78
Chapter 3.2 | Figure 3 Prevalence of myopia (a), grades of myopia (b), hyperopia (c) and astigmatism (d) according to age 80
Chapter 3.3 | Figure 1 Prevalence of myopia against age stratified by decade of birth 89
Chapter 3.3 | Figure 2 Prevalence of myopia as a function of age for two birth cohorts (1910-1939, 1940-1979) 89
Chapter 3.3 | Figure 3 Prevalence of myopia stratified by highest educational level achieved 90
Chapter 3.3 | Figure 4 Distribution of highest educational level achieved stratified by year of birth 90

Chapter 3.3 | Figure 5 Myopia prevalence by birth cohort and educational level in individuals aged 45 to 65 years 91

Chapter 3.4 | Figure 1 Graph showing distribution of refractive error 99

Chapter 3.4 | Figure 2 Scatter plot of age at myopia onset (<40 years old) and myopia severity 100

Chapter 3.4 | Figure 3 Prevalence of myopia among participants of 1998-1999 examinations and 2008-2010 examinations against age at refraction 100

Chapter 4 Environmental factors for myopia

Chapter 4.2 | Figure 1 Cohort prevalence of myopia by age 116

Chapter 4.2 | Figure 2 Predictors for myopia from the overall life course analysis 128

Chapter 4.2 | Figure 3 Receiver operating characteristic curves for prediction of myopia 129

Chapter 4.2 | Figure 4 Association between myopia and cognitive ability over childhood 132

Chapter 4.3 | Figure 1 Flowchart of inclusion of study participants 142

Chapter 4.3 | Figure 2 Association of all-day ultraviolet B (UVB) exposure at different ages with myopia 143

Chapter 5 Genetic factors for myopia and the interplay with environmental risk factors

Figure 5.1 Path diagram and matrices for the variance and covariance of a trait in a twin pair 152

Figure 5.2 Scatter plots of spherical equivalent for MZ and DZ pairs 153

Figure 5.3 Q-Q plot for the association between all SNPs analysed and spherical equivalent in TEDS 156

Figure 5.4 Manhattan plot of the GWAS for refractive error in TEDS 157

Figure 5.5 Regional plot of the loci associated on chromosome 11 158

Figure 5.6 Results of power calculations for GWAS in TEDS given difference effect sizes and minor allele frequencies 160

Figure 5.7 Genetic association significance levels for spherical equivalent and age of spectacle wear with previously identified SNPs in myopia 161

Figure 5.8 Lowess scatter plot of correlation between spherical equivalent and age of spectacle wear 162

Figure 5.9 Correlation between spherical equivalent and reported scale of poor distance vision for all individuals and for those who reported glasses wear 162

Chapter 5.4 | Figure 1 Scatter plot of refractive error against intelligence with linear regression line 166

Chapter 5.4 | Figure 2 Bivariate twin ACE model for refractive error and intelligence 167
Chapter 5.4 | Figure 3 Genome-wide polygenic scores for intelligence (A) and refractive error (B) respectively predict variance in refractive error and intelligence 168
Chapter 5.5 | Figure 1 SNPs associated with early-onset and later-onset effects on refractive development during childhood 178
Chapter 5.5 | Figure 2 Association between a genetic risk score for 39 SNPs and refractive error trajectories in ALSPAC participants 179
Chapter 5.5 | Figure 3 Refractive error trajectories in ALSPAC participants for SNPs showing evidence of an interaction with near work or time outdoors 181
Chapter 5.5 | Figure S1 Meta-analysis summary plots for cross-sectional cohorts 198
Chapter 5.5 | Figure S2 SNP effects in European and Asian meta-analysis samples 201
Chapter 5.5 | Figure S3 SNP x near work interactions at ages 7 and 15 in the ALSPAC discovery cohort 202

Chapter 7 Materials

Figure 7.1 Progress of refractive error data collection 227
Figure 7.2 Histogram of spherical equivalent 230
Figure 7.3 Distribution of refractive error by age 230
Figure 7.4 Prevalence of myopia and hypermetropia by age at refraction 233

List of Tables

Chapter 1 Introduction

Table 1.1 Definitions of lesions for proposed classification of Myopic Maculopathy 28
Table 1.2 Estimated prevalence of myopia by age and ethnicity 34
Table 1.3 Myopia prevalence in children of white European ethnicity in UK based studies 35
Table 1.4 Myopia prevalence among university students 1871-1985 38
Chapter 1.52 | Table 1 Summary of published GWAS in myopia 59

Chapter 3 The changing epidemiology of myopia in Europe & the UK

Chapter 3.2 | Table 1 Description of the 15 European Eye Epidemiology consortium studies included in this meta-analysis of refractive error 76
Chapter 3.2 | Table 2 Prevalence of myopia, hyperopia and astigmatism stratified by age 79
Chapter 3.2 | Table 3 Prevalence of myopia, hyperopia and astigmatism stratified by age and gender 81
Chapter 3.3 | Table 1 Description of the 15 European Eye Epidemiology consortium studies included in this meta-analysis of refractive error 88
Chapter 3.3 | Supplementary Table 2 Prevalence of myopia against birth year stratified by age 95
Chapter 3.4 | Table 1 Distribution of myopia by age at onset and severity 99

Chapter 4 Environmental factors for myopia

Chapter 4.2 | Table 1 Preconception factors 118
Chapter 4.2 | Table 2 Prenatal, perinatal and postnatal factors 120
Chapter 4.2 | Supplementary Table 1 Preschool factors 122
Chapter 4.2 | Supplementary Table 2 Childhood factors 125
Chapter 4.2 | Table 3 Adolescence factors 127
Chapter 4.3 | Table 1 Characteristics of participants with and without myopia 142
Chapter 4.3 | Table 2 Association of ultraviolet B radiation exposure, education, serum vitamin D concentrations and lutein concentrations with myopia 143
Chapter 4.3 | eTable Association between myopia and genetic variants associated with vitamin D metabolic pathway 147

Chapter 5 Genetic factors for myopia and the interplay with environmental risk factors

Table 5.1 Model-fitting results for univariant analysis of spherical equivalent 154
Table 5.2 The association results in TEDS for known genetic loci for spherical equivalent in adult GWAS 159
Table 5.3 Comparison of the association between cognitive score 'g' and the eyesight questionnaire and spherical equivalent 163
Chapter 5.4 | Table 1 Twin participant characteristics by zygosity, myopic status and genotype availability 166
Chapter 5.5 | Table 1 Demographics of study samples 176
Chapter 5.5 | Table 2 Age-of-onset of SNP associations with refractive error in the 177

discovery cohort
Chapter 5.5 | Table 3 Replication meta-analysis results for SNP main effects 180
Chapter 5.5 | Table 4 Summary of findings 182
Chapter 5.5 | Table S1 SNPs examined 190
Chapter 5.5 | Table S2 Age-of-onset of SNP associations in discovery cohort 191
Chapter 5.5 | Table S3 Meta-analysis of SNP x near work interaction effects in cross-sectional cohorts 192
Chapter 5.5 | Table S4 Meta-analysis of SNP x time outdoors interaction effects in cross-sectional cohorts 193
Chapter 5.5 | Table S5 Genotyping and imputation details 194
Chapter 5.5 | Table S6 Time spent performing near work 194
Chapter 5.5 | Table S7 Time spent outdoors 195
Chapter 5.5 | Table S8 Refraction details for the ALSPAC discovery cohort 196

Chapter 6 Discussion and conclusions
Table 6.1 Genomic regions influencing more than six phenotypes including myopia 220

Chapter 7 Materials
Table 7.1 TEDS demographic representation during the life course 225
Table 7.2 Refractive classification definitions 228
Table 7.3 Representativeness of TEDS Myopia Study responders 229
Table 7.4 Prevalence of refractive error in TEDS Myopia Study 232

Abbreviations

1958 BBC	1958 British Birth Cohort
A	Additive genetic factors
AIC	Akaike information criterion
AL	Axial length
ALIENOR	Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes Study
ALSPAC	Avon Longitudinal Study of Parents And Children
AOSW	Age of Spectacle Wear
AUC/AUROC	Area Under Receiver Operator Curve
β / Beta	Beta coefficient
BATS	Brisbane Adolescent Twin Study
BDES	Beaver Dam Eye Study
BMES	Blue Mountains Eye Study
BMI	Body Mass Index
C	Common environmental factors
CHASE	Child Heart and Health Study in England
CI	Confidence Interval
CLEERE	Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error study
CR	Corneal radius
CREAM	Consortium for Refractive Error and Myopia
D	Dioptres
D	Dominant genetic factors
DALYS	Disability-adjusted life years
df	Degrees of freedom
DNA	Deoxyribonucleic Acid
DZ	Dizygotic (twins)
E	Unique environmental factors
E^3	European Eye Epidemiology consortium
EPIC-Norfolk	European Prospective Investigation into Cancer Norfolk study
ERF	Erasmus Rucphen Family
g	g' factor (general cognitive ability)
GEM	GEnes in Myopia study
GHS	Gutenberg Health Study
GUSTO	Growing Up in Singapore Towards healthy Outcomes study
GWAS	Genome-wide association study
GZT	Guangzhou Twin eye Study
h^2	Heritability due to additive +/- dominant genes
IQ	Intelligence Quotient
KORA	Kooperative Gesundheitsforschung in der Region Augsburg study
LL	Log-likelihood
MAF	Minor Allele Frequency
META-PM	META_analysis for Pathologic Myopia study group
Montrachet	Maculopathy Optic Nerve nuTRition neurovAsCular and HEarT diseases and
3C	Three-City study

MZ	Monozygotic (twins)
NHANES	National Health And Nutrition Examination Survey
NICER	Northern Ireland Childhood Errors of Refraction study
OR	Odds Ratio
p	P value
POLA	Pathologies Oculaires Liées à l'Age
QQ	Quintile-Quintile plot
r	Correlation coefficient
SAVES	Sydney Adolescent Vascular and Eye Study
SCORM	Singapore Cohort Study of the Risk factors for Myopia
SD	Standard Deviation
SE	Spherical Equivalent
SES	Socioeconomic Status
SMS	Sydney Myopia Study
SNP	Single Nucleotide Polymorphism
STARS	Strabismus, Amblyopia and Refractive Error in Singaporean Children
TEDS	Twins Early Development Study
TES	Thessaloniki Eye Study
TEST	Twins Eye Study Tasmania
TwinsUK	TwinsUK cohort
UK	United Kingdom
US/USA	United States of America
UVB	Ultraviolet B radiation
V	Variance
WESDR	Wisconsin Epidemiological Study of Diabetic Retinopathy
WHO	World Health Organisation
X^2	Chi-squared statistic

Chapter 1 | Introduction

1.1 Overview

Myopia is the most common eye condition worldwide and the leading cause of correctable visual impairment. The optical properties of myopia are well understood and methods to correct the focus of light onto the retina are effective, albeit not widely accessible in the developing world. Genetic and environmental factors play key roles in the risk of an individual developing myopia. However, despite extensive research in the field, there is a currently neither sufficient genetic information nor environmental measures that can confidently predict who will develop myopia. This will become increasingly relevant as the global prevalence of myopia rises and treatments to target both onset and progression of the condition are developed.

In this chapter I will provide an introduction to what myopia is, how it is defined, the natural history of the condition, how it is corrected, and why it continues to place an individual at risk of visual impairment despite refractive correction. I will review the current epidemiology of the condition in both adult and paediatric populations, with particular attention paid to the evidence for a rising global prevalence and the potential implications of this for the future. I will discuss what is known about key environmental associations with myopia in turn. Finally, I will review our current understanding of the genetic architecture of myopia from twin studies, linkage studies, genome-wide association studies and functional work.

1.2 Definition and Clinical Aspects of Myopia

1.21 What is myopia?

Myopia, commonly called short sightedness in the United Kingdom and near sightedness in the United States, is a form of ametropia where the eye incorrectly focuses parallel light to a point anterior of the retinal plane. This may be due to inappropriate refraction of incipient light at the corneal surface or lens, or increased axial length of the eye, illustrated in Figure 1.1. In a normal length eye, the increased dioptric power of the lens or cornea is the cause of excessive refraction of light – this is generally termed *refractive* or *index* myopia (1). Typical examples of the latter are keratoconus, where the corneal refractive power increases with progressive ectasia of the cornea, and cataract development ('lens induced myopia'), where the lens becomes increasingly hard (or 'sclerotic') and the refractive power is similarly increased. However, the majority of myopia is a result of axial elongation of the globe, what is termed *axial* myopia, and this will form the focus of this thesis.

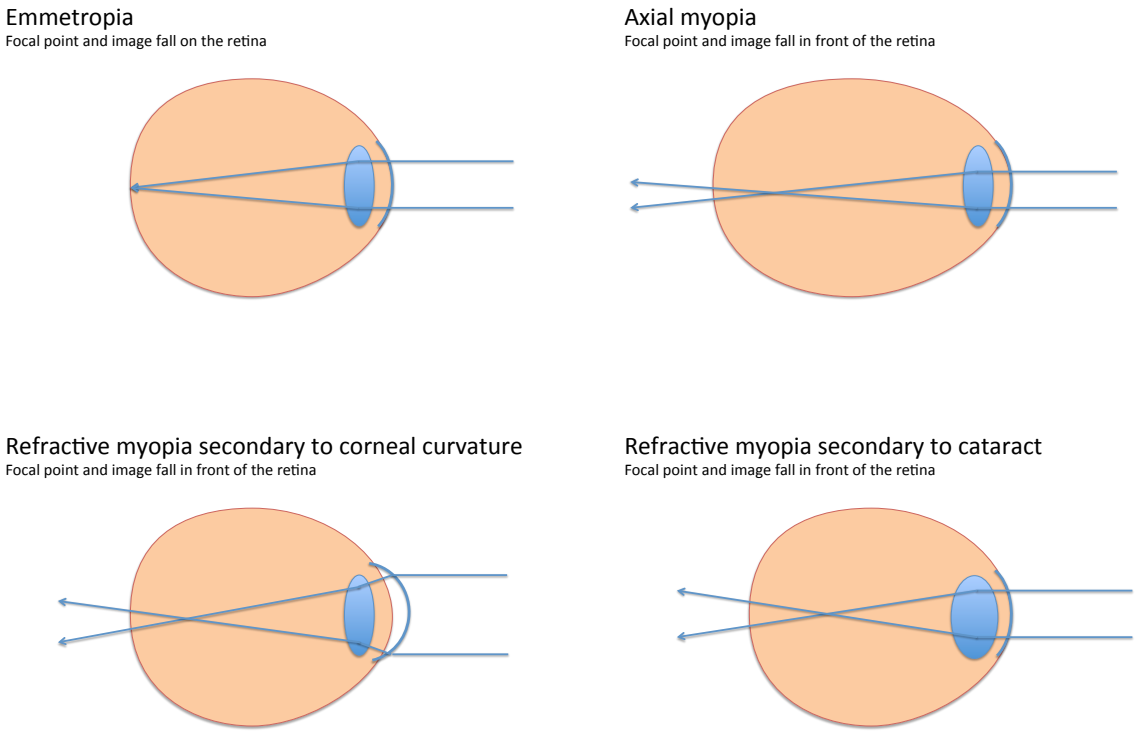


Figure 1.1 Emmetropia, axial myopia, refractive myopia secondary to corneal curvature and refractive myopia secondary to cataract

1.22 Definitions of myopia and natural history

Myopia is generally defined by the dioptric power of a concave lens required to focus light accurately onto the retinal plane [Figure 1.2]. This is reported by calculation of the spherical equivalent (SE) measured in dioptres (D) - using the standard formula of *spherical lens + (cylindrical lens/2).* The degree or amount of spherical equivalent can also be used to grade myopia. However, spherical equivalent cut-offs used to define myopia are rather heterogeneous, particularly between adult and paediatric studies; presence of myopia is variably defined as < 0 D to < -1.50 D (2-4), The grade of myopia is also variable with some authors using a binary division of low ≤ -1.00D and high ≤ -5.00 D, and others using a three-tier division of low (≤ -0.75 D), moderate (≤ -3 D) and high (≤ - 6 D) (5, 6). This variation leads to inherent difficulties in comparison of myopia prevalence and associations between different cohorts.

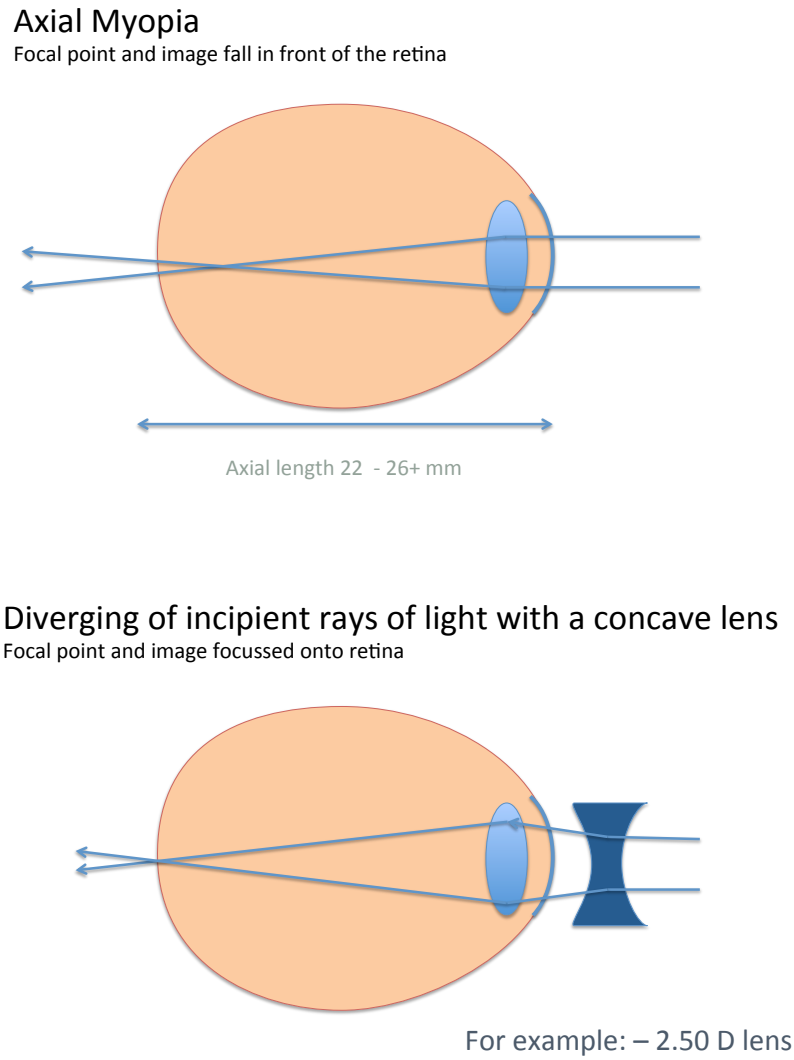


Figure 1.2 Concave lenses are used to diverge incipient rays of light in a myopic eye to ensure light is focussed onto the retinal plane

Myopia can also be defined in terms of age at onset – '*childhood, juvenile, early or school* onset', and '*adult*' onset (7-11). The majority of myopia develops during childhood, with progression during adolescence and early adulthood. In an older adult British population the median age of myopia onset was 15 years (12), as discussed in Chapter 3.4 and Chapter 5.3, whilst in more recent studies of Finnish and Singaporean children the median age of myopia onset was earlier at 11.3 and 8.8 years respectively (13, 14). The eye grows rapidly from birth until the age of around two; at one week old the eye is typically 17 mm in length, by three months it is 18 mm, by four months it is 19.5 mm, by 12 months it is 20 mm, and at two years the eye is around 21 mm long with the cornea generally reaching full size (10.6 x 11.7 mm) (15, 16). At this point the cornea contributes approximately two-thirds of the refractive power of the eye (~43D), as

determined by Snell's Law which states that the power is equal to the difference between two refractive indices (in this case the derived refractive index of the full thickness of cornea which is 1.3375 and the refractive index of air which is 1.00) (17). This period represents the period of emmetropisation when the typical hypermetropia associated with infancy reduces. By the age of 10 most eyes have reached near adult size with an axial length of 23± 0.91 mm, a lens approximately 3.50 mm in thickness, which contributes 21±1.5 D to the dioptric power of the eye (18). However, particularly in the case of axial myopia, scleral remodeling can continue during adolescence and early adulthood (19). Most experience stabilization of refractive error during early adulthood followed by a hypermetropic shift with increasing age (20). This is followed by a myopic shift in older age, approximately after the age of 65 in the Blue Mountains Eye Study, the majority of which is due to the development of nuclear sclerotic cataracts (21). In the Beaver Dam Eye Study the severity of nuclear sclerosis was strongly related to change in refractive error; those with mild nuclear sclerosis at baseline had a change of +0.35D compared to a change of -0.53D in those with severe nuclear sclerosis over a ten-year period (22). The authors performed additional analyses where they adjusted for the effect of 'lens induced myopia' (by adjustment for severity of nuclear sclerosis at baseline) and found individuals over the age of 70 continued to display a significant myopic shift. Whilst the mechanism for this remains unclear it is almost certainly not due to changes in axial length or corneal power, as these remain fairly stable after the age of 40 (23). There is evidence for other 'lenticular changes' in later life - the lens has been found to get thicker and more steeply curved with age in two studies examining ocular biometry in-vivo and in isolated human eye-bank lenses (24, 25). However, although both attempted adjustment for lens opacity, they acknowledged these changes might still represent undetectable, pre-cataract changes in the lens.

Childhood onset myopia is the focus for current research due to the fact that associations with myopia, potentially modifiable targets such as time outdoors, are generally thought to affect the growth of maturing eye. It is also relevant to researchers that those developing myopia at a younger age are more likely to develop high myopia (14), with increased risk of sight threatening complications in adulthood; onset before the age of nine in the UK predicts high myopia in adulthood with 73% specificity and 80% sensitivity (26). Children aged 6-11 years with a family history of myopia, and therefore highly likely to become myopic, have axial elongation of the globe that may

precede the development of a myopic spherical equivalent (27). In both animals and humans, early neonatal visual experience has effects on eye growth and development of refraction (28, 29) (14, 30). Refractive error at age 6-11 years has been identified as the single best predictor of future myopic status (11). Even in very young children the risk of future myopia appears to be ascertainable by early measures of refraction; in a prospective study of 77 children aged 6 to 12 months, followed to the age of 15 years, those in the lower half of the distribution of refractive error were 4.33 times (95% CI 1.66 – 11.36, p=0.005) more likely to develop myopia than those in the upper half of the distribution (31). These findings are limited to juvenile onset myopia, given that those developing myopia after the age of 15 years would not be identified, and arise in a very small sample but the refractive trend between <1 year old to 15 years old remains interesting.

However, a significant proportion of the population, ranging from 27% up to 76%, will have 'adult onset' myopia, variably defined as developing after the age of 17, 18, or 21 years (30, 32, 33). This observation, with important implications in terms of misclassification of future myopic status, was replicated in my own research (see Chapter 3.4). Reasons why individuals develop myopia in adulthood are not well understood but lifestyle and occupations which involve long periods of near work have been implicated (34).

Ocular biometry can be used to categorise myopia – this is particularly useful for early onset myopia that is characteristically associated with excessive axial length. One can either use the simple measure of axial length, as this is correlated with refractive error and proved successful as a proxy in epidemiological and genetic association studies (35, 36), or examine the mismatch of optical effects between different biometric refractive components. This is typically performed by calculating the ratio between the axial length (AL) and corneal radius (CR). The AL/CR ratio has a high correlation with refractive error (37, 38). One advantage of this technique is that dilation with cycloplegia is not required.

A final method of classification employed by some studies is the use of unaided distance visual acuity, often utilised when refractive error data is not available. An approximate relationship between unaided visual acuity and myopia is used clinically; a visual acuity of 6/12 Snellen has been reported to correlate with a refraction of

approximately -0.66D, whilst a visual acuity of 6/18 Snellen approximately correlates with –1 D of myopia (39). In a paediatric population, a cut-off point of ≤ - 0.75 D had a sensitivity and specificity of 91.8% and 93.7% respectively to predict an uncorrected visual acuity of LogMAR > 0.3 (either eye) (40). However, although the specificity of estimating lifetime risk of myopia given a reduced uncorrected visual acuity at 16 was high (91%) in the 1958 British Birth Cohort, it was found to have a low sensitivity for identifying all those who later became myopic (16% for all myopia, 69% for severe myopia) (30).

1.23 Refractive correction of myopia

Myopia is corrected by diverging incipient rays of light, before they enter the eye [Figure 1.2]. This is achieved through the use of spectacles, contact lenses, intra-ocular lenses, or, more recently, remodelling of the corneal surface using an ablative laser in laser refractive surgery. These clinical services are readily available in developed countries – however there are potential complications from contact lens use and laser refractive surgery, and significant financial expense to both the individual and health care services.

In less developed countries visual impairment as a result of uncorrected refractive error, including myopia, remains a problem; the World Health Organization (WHO) has identified 153 million visually impaired people due to uncorrected refractive error (41). This has significant implications for childhood development, education, employment and overall productivity. The Global Burden of Disease Study 2010 measured disease burden using disability-adjusted life years (DALYS), which is equal to the sum of years of life lost plus years lived with disability as a result of a disease. Refractive disorders were estimated to account for 81 DALYS (per 100,000), which was a 19.3% increase on 1990 (42). The identification and treatment of myopia is now a WHO priority within their initiative to eliminate avoidable blindness (41) and myopia is a priority of VISION 2020, the international initiative against visual impairment (43). In Europe uncorrected refractive error is less common, however a systematic analysis of vision loss in 2010 identified that the second most common identifiable cause of blindness in Western Europe was uncorrected refractive error; proportionally 14% of vision loss was attributable to uncorrected refractive error, whilst the most common identifiable cause was macular degeneration at 16% (44). In the UK EPIC-Norfolk Eye Study, participants aged 48-89 years, refractive error was common but uncorrected

refractive error was only present in approximately 2% (defined as ≥1 line improvement with pinhole-correction in the better eye in participants with LogMar presenting visual acuity <0.3) (45).

1.24 Pathological complications of myopia

Myopia, despite refractive correction, places an individual at an increased risk at a number of sight-threatening diseases, namely glaucoma (open angle glaucoma), cataract (nuclear, cortical and posterior subcapsular), retinal tears which may lead to a retinal detachment, and myopic maculopathy or myopic macular degeneration (46, 47). The incidence of these complications is greater in those with high myopia, but they are also seen in the more common low myopia. As illustrated in the forest plot below [Figure 1.3] from two studies, the odds of a retinal detachment increases from approximately OR 3-4 in low myopia to OR >20 with high myopia.

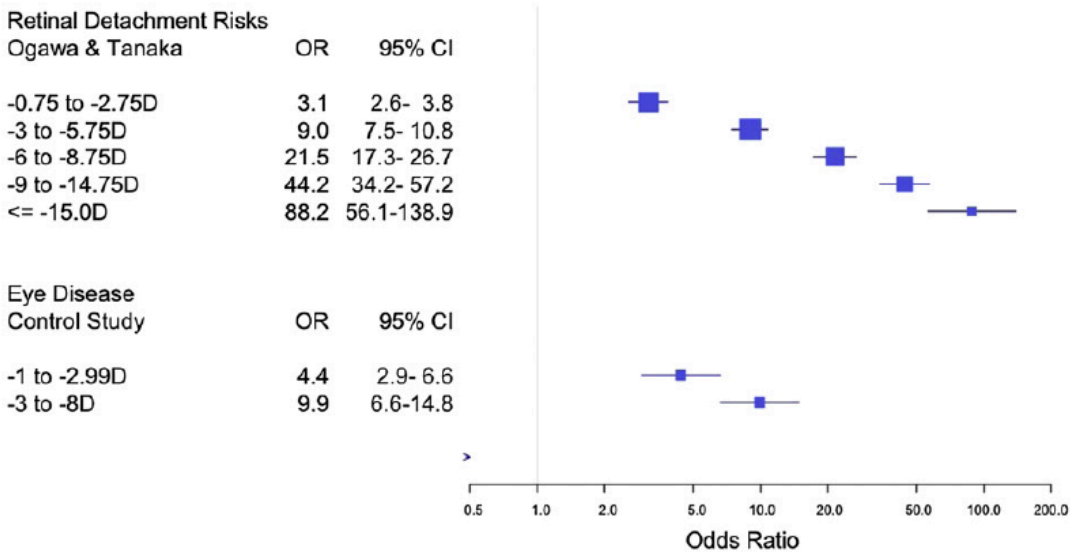


Figure 1.3 Odds ratios for retinal detachment associated with different grades of myopia, reproduced with permission from (48, 49)

The myopic eye undergoes changes as a result of the axial elongation, and high myopia is associated with distinct patterns of pathologic myopia or myopic maculopathy. There have been a number of proposed classification systems incorporating features of advanced myopia such as posterior staphyloma, lacquer cracks, chorioretinal atrophy and Fuchs spot, illustrated in Figure 1.4.

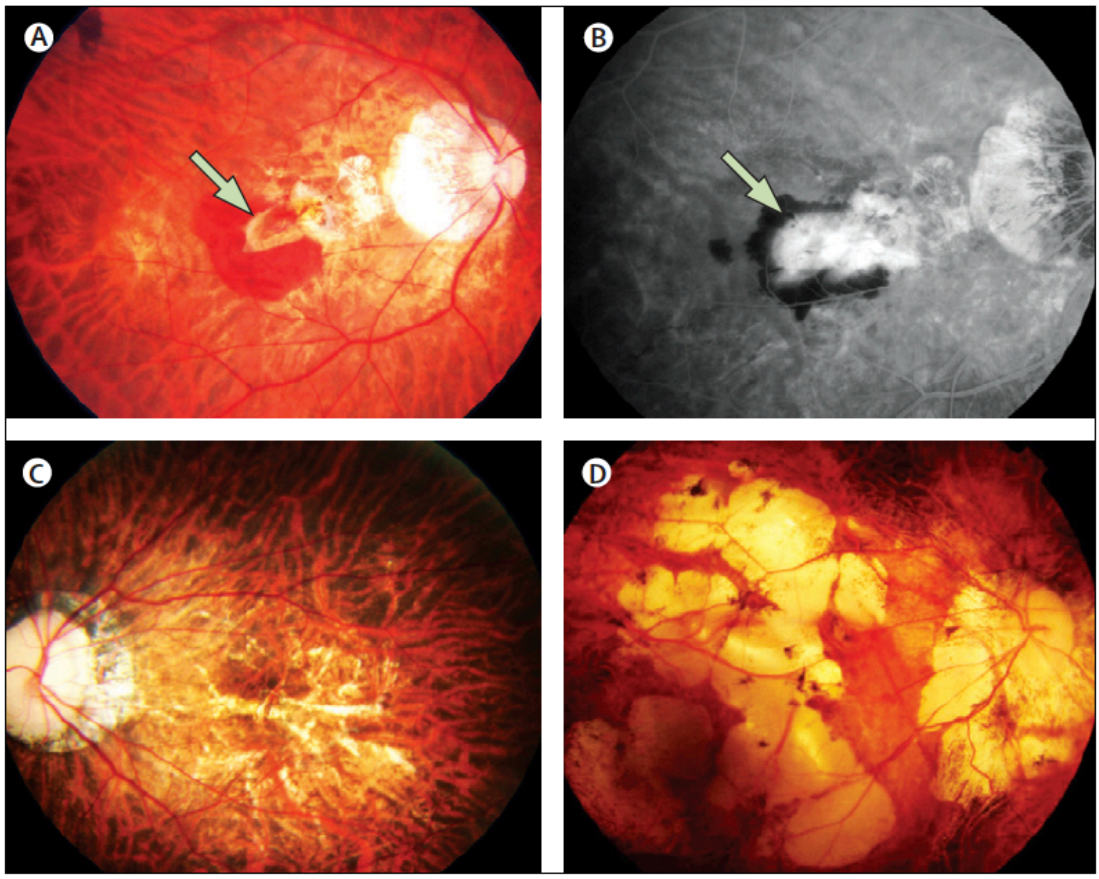


Figure 4: **Various fundus lesions specific to pathological myopia**
(A, B) Myopic choroidal neovascularisation (A, colour fundus photograph; B, fluorescein fundus angiogram).
(C) Lacquer cracks. (D) Myopic chorioretinal atrophy.

Figure 1.4 Features of pathological myopia, reproduced with permission from (50)

Most recently, a classification system has been developed and evaluated by the META_analysis for Pathologic Myopia (META_PM) Study Group (51) incorporating elements of previous classification systems (52-54). Five categories of myopic maculopathy were proposed (Category 0: No macular lesion, Category 1: Tessellated fundus, Category 2: Diffuse chorioretinal atrophy, Category 3: Patchy chorioretinal atrophy, Category 4: Macular atrophy) with additional 'plus' features (lacquer cracks, choroidal neovascularisation and Fuchs spot), detailed in Table 1.1. Posterior staphyloma was defined on a 're-categorised and simplified' Curtin's classification (52). As shown Table 1.1, a staphyloma was defined to occur if there was local bulging of the sclera that has a radius of less than the surrounding curvature of the wall of the eye, and the classification was based on the involvement of the macula, the size, and the proximity to the macula and optic disc. The classification system showed a good level of intra-observer agreement (weighted kappa statistic ≥0.6) and inter-observer

agreement (kappa statistic 0.5-0.8) but the classification system is yet to appear in routine use in published literature.

I. Myopic macular lesions		
Category 1	**Tessallated fundus**	Well defined choroidal vessels that can be observed clearly around the fovea as well as around the arcade vessels
Category 2	**Diffuse chorioretinal atrophy**	Yellowish white appearance of the posterior pole. When present, further estimate size and extent using disc area as a relative size unit
Category 3	**Patchy chorioretinal atrophy**	Well-defined, grayish white lesions in the macular area or around the optic disc
Category 4	**Macular atrophy**	Well-defined, grayish white or whitish, round chorioretinal atrophic lesion in the foveal region; may appear around a regressed choriodal neovascularisation (Fuchs spot)
II. "Plus" lesions of the myopic macular lesions		
Lacquer Cracks		Yellowish linear lesions in the macula. Often criss-cross over the underlying choroidal vessels. Newly developed laquer cracks may be seen with haemorrhage
Chorioretinal neovascularisation		Lesion associated with choroidal neovascularisation and exudation, haemorrhage or serous retinal detachment at the posterior pole
Fuchs spot		Pigmented grayish white scar of myopic choroidal neovascularisation without associated exudation, and sometimes associated pigmentation
III. Posterior Staphyloma		
Local bulging of the sclera at the posterior pole of the eye, that has a radius of less than the surrounding curvature of the wall of the eye: - **Macula involved: Wide (Curtin's type I), Narrow (Curtin's type II), and inferior (Curtin's type V)** - **Macula not involved: peripapillary (Curtin's type III), nasal (Curtin's type IV) and inferior (Curtin's type V)** - **Others (Curtin's type VI-X and posterior staphyloma that cannot be classified into Curtin's I-X)**		

Table 1.1 Definitions of lesions for proposed classification of Myopic Maculopathy, adapted from (51).

The risk of myopic maculopathy also sharply rises with increasing myopia; as illustrated in Figure 1.5, the odds of myopic maculopathy increases from OR 2.2 with low myopia to OR >40 with high myopia.

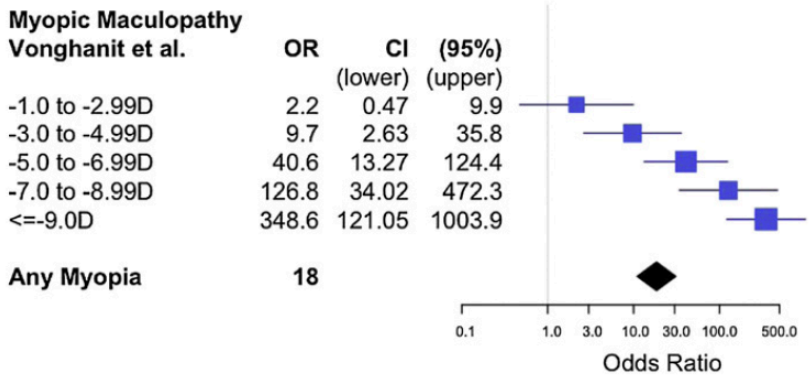


Figure 1.5 Forest plot of odds ratios for myopic maculopathy with increasing myopia, reproduced with permission from (48, 55)

Sight loss, especially of the central vision, is associated with advanced myopic maculopathy (category 4) and in the atrophic form there is currently no available treatment. Therefore, there is concern that with the increasing myopia prevalence the current estimate of 12.2% (56) to 14.2% (57) of visual impairment in working age adults attributable to this condition could rise. In a meta-analysis of five studies from the Netherlands, the cumulative risk of visual impairment in later life was 5.7% in emmetropes but 39% for those with high myopia (35).

1.3 Epidemiology of Myopia

The epidemiology of myopia has been extensively reviewed by others (4, 7, 58, 59). I will select relevant papers for specific mention to provide an introduction to my research.

1.31 Epidemiology of myopia in adults

The prevalence of myopia is substantially higher in urban Asian populations, with less myopia in western countries, and low levels identified in rural areas and low-income countries. Adult population-based studies in the early part of the 21st century have identified 40-50% prevalence in many Asian countries, including 41.8% in Japan (60), 48.1% in Indonesia (61), 42.7% in Myanmar (62), and 38.7% in Singapore (63).

However, the prevalence of myopia in young adults in these countries is substantially higher and reaching epidemic proportions; estimated prevalence in 20 year-olds in Hong Kong, Taiwan, Singapore and South Korea was approximately 60% in 1990 and 80% in 2010 (64), whilst in a publication from Taiwan in 2009, 95.9% of University students were myopic (65).

It appears the highest levels of prevalence are in young adults of Chinese ancestry; in a review from the Lancet in 2012 Morgan et al decomposed the dramatic rise in myopia prevalence into three time periods and three ethnicities within Singapore, an urban Asian city with very high levels of myopia (50). Between 1987 and 2010 the prevalence of myopia rose from around 25% to 70% in those of Malay background and to an even greater extent in individuals of Chinese ethnicity (from approximately 50% to 85%) (50, 66-70), illustrated in Figure 1.6. A high myopia prevalence is seen in individuals of Chinese ethnicity even when they are not living in East Asia; in a US multi-ethnic study the prevalence of myopia was 14.2% in Hispanic participants, 21.5% in Black participants, 31.0% in White participants, and 37.2% in Chinese participants (71).

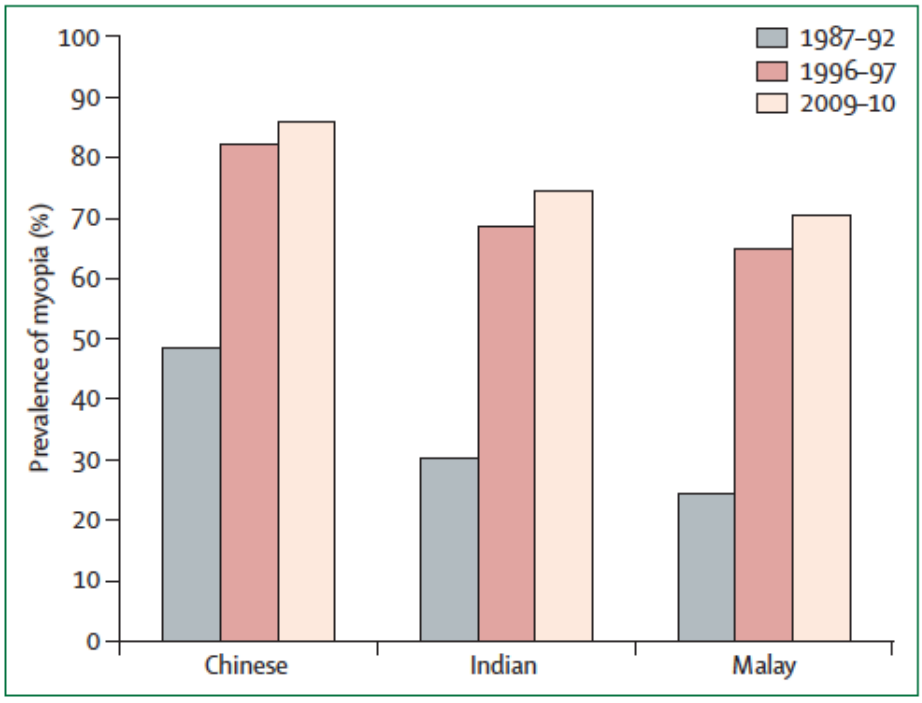


Figure 1.6 Changes in the prevalence of myopia in the three major ethnic groups in Singapore, reproduced with permission from (50)

In comparison the prevalence of myopia in rural, low-income countries remains low. In the South Pacific Solomon Islands myopia prevalence was 0.8% in the 1960s (72), in Malawi a prevalence of 2.5% was identified in 1995 (73), in a Nigerian population in 2007 prevalence was 16.2% (74), and in a South African population 11.4% were documented to have myopia in 2016 (75). Although illustrative, these aforementioned studies' age variations make none of these estimates directly comparable.

In western countries, access to historical cohorts provides an account of the changing epidemiology of myopia. The literature indicates that low levels of myopia in the 19th century, particularly in rural areas, were followed by increasing myopia rates in the early part of the 20th century. In 1882 and 1884 the myopia prevalence was 2.4% in a farm labourer sample from Denmark and Germany respectively (76, 77). In Germany in 1928 the general adult myopia prevalence was higher at 13.7% (78); similarly in the US the prevalence was 18.9% in 1932 (79) and 17.7% in 1950 (80). Estimates in the early part of the 21st century in young adults (aged 20-29) in the US in 1932 range from 19.6% (81) to 22.0% (79).

More recent cohorts estimate the prevalence of myopia at 35.0% in young adults in Norway (82), 15.0% in the Australian Blue Mountains Eye Study (BMES) (83), and 33.1% in the US National Health and Nutrition Examination Survey (NHANES) (84). Similar figures were obtained by the US Beaver Dam Eye Study, which showed a high prevalence of 42.9% in 43-54 year olds reducing to 14.4% in those over 75 (85). Higher prevalence of myopia in young individuals could be attributable to two factors; longitudinal changes in refractive error and cohort effects. In the Beaver Dam Eye Study over 2000 individuals were followed up over a ten-year period; in the younger adults there was a hyperopic shift and in the older adults there was a myopic shift, largely attributable to nuclear sclerosis (22). However, the degree of myopic shift in later life was small (mean -0.10 D over ten years in individuals aged 70+) and a far greater effect was seen with decade of birth (mean refraction aged 55-59 was 0.20D in those born 1928-1932 compared to -0.5D in those born 1938-1942) (22). The Eye Disease Prevalence Research groups sought to create age-specific estimates of refractive error prevalence for three geographical regions using six population based studies and provide reliable estimates of the current burden of refractive error in grouped populations (6). The estimated crude prevalence of myopia (defined as ≤ -1.00 D) was 25.4%, 26.6% and 16.4% in the US, Western Europe and Australia respectively.

However, evidence of rising myopia levels in Western countries has been published; in Finland myopia prevalence was <10% in the first three decades of the 20th century increasing to 21-30% in those born in the second half of the 20th century (86), in the US myopia prevalence increased from 25.0% to 41.6% in adults between ~1970 and ~2000 (3), and in a young Israeli population myopia prevalence rose from 20.3% to 28.3% between 1990 and 2002 (87). I have explored evidence for similar rising trends in Western Europe in Chapter 3.3.

Estimates of adult myopia prevalence in the UK include the aforementioned figure of 26.6% obtained from the Western Europe sub-analysis of the Eye Disease Prevalence Research Group meta-analysis (participants aged 40+ years), the 1958 British Birth cohort estimate of 49% (participants aged 44 years), and most recently the 2009 UK Biobank myopia prevalence of 27% (participants aged 40-69), although the latter does not comprise a truly UK-representative population sample (6, 9, 88).

1.32 Epidemiology of myopia in children

The prevalence of myopia in children shows similar trends with the lowest levels in non-urbanised, low-income countries and the highest levels in urban, higher income Asia countries. Studying myopia rates in children also provides some insight into the likely burden of adult myopia in the future. Recent studies of comparative but broad age groups (5-15 years) estimated 1.2% myopia prevalence in Nepal (89), 4.1% in India (90) and 2.9% in in the South Pacific Vanuatu Islands (91), with higher levels of 20.7% in Malaysia (92), 38.1% in China (93) and 36.7% (NB: aged 6-9 years) in Singapore (94). In children from Singapore a high prevalence of 29% and 51% in primary school and 9 year-old samples respectively was seen (94-96). Dramatic increases in myopia prevalence in urban Asia are seen in children. In Taiwan the rise has been particularly well documented, as illustrated in Figure 1.6. In the year 2000 in Taiwan it was estimated that 50% of 9 year olds, a near 40% increase on the estimate 17 years prior, and up to 95.9% of university freshmen are now myopic (65, 97).

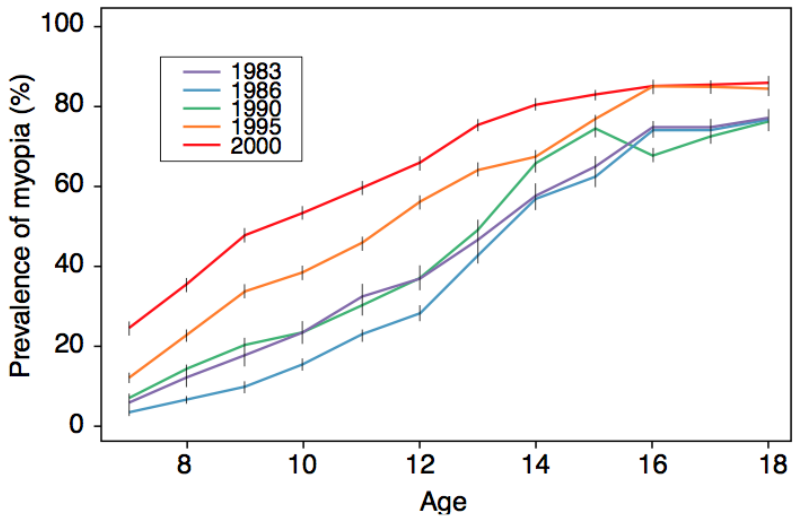


Figure 1.7 Prevalence of myopia in school children in Taiwan across five examination periods, adapted from (7, 97)

In western populations age-specific prevalence estimates are lower than those seen in urban Asia; for example 12% in 11.9 year-olds in the Australian Sydney Myopia Study (98) and 20.0% in 12 year-olds in the US Orinda Longitudinal Study (99). However, with the increasing multi-ethnicity of countries, it is perhaps best to consider myopia prevalence by ethnicity. A systematic review published in 2016 followed this rationale; their findings, illustrated in Table 1.2 below, confirm the dramatically higher levels of myopia seen in East Asian children. They also highlight that whilst there are differences in prevalence by ethnicity, the country of residence is also highly influential; as illustrated by the low prevalence in children of black ethnicity living in Africa compared to the higher prevalence in those living outside of Africa, and similarly in children of East Asian ethnicity living in Singapore or Taiwan compared to Australia.

Ethnicity	Prevalence (%) of myopia by age			
	5 years	10 years	15 years	18 years
White	1.6 (1.0 - 2.5)	6.7 (4.1 – 10.3)	16.7 (10.6 - 24.5)	22.8 (14.6 – 32.7)
East Asian	6.3 (4.4 – 9.2)	34.5 (26.7 – 44.0)	69.0 (60.6 – 76.8)	79.6 (73.0 – 85.4)
East Asian in Australia	1.9 (0.8 – 4.2)	13.6 (6.2 – 26.5)	40.6 (22.3 – 60.9)	
East Asian in Singapore	14.9 (9.9 – 22.4)	59.0 (47.2 – 70.2)	86.2 (79.4 – 91.1)	91.7 (87.2 – 94.8)
East Asian in Taiwan	10.1 (5.9 – 19.8)	48.0 (34.0 – 67.4)	80.0 (69.0 – 90.0)	87.6 (79.9 – 94.0)
South Asian	5.3 (2.9 – 9.6)	9.2 (5.2 – 15.7)	13.0 (7.4 – 21.6)	13.9 (7.7 – 23.5)
South-East Asian	6.7 (2.9 – 14.4)	11.5 (5.3 – 23.3)	23.7 (11.7 – 41.8)	28.0 (13.8 – 48.2)
Black in Africa	2.8 (1.5 – 5.0)	1.8 (1.1 -2.7)	5.5 (3.1 – 9.0)	
Black not in Africa	4.8 (4.0 – 5.7)	8.2 (6.8 – 9.8)	19.9 (14.3 – 26.5)	
Middle Eastern or North African	3.5 (2.0 -5.7)	5.5 (3.4 – 8.8)	19.6 (12.8 - 28.6)	47.1 (34.2 – 60.4)

Table 1.2 Estimated prevalence of myopia by age and ethnicity.
Prevalence estimates are medians (95% credible intervals in parenthesis) of the posterior distributions for predicted prevalence from the Bayesian multilevel binomial logistic regression of the log odds of myopia adjusting for ethnic specific associations with age, ethnic specific associations with survey years (where possible) and environmental setting. Adapted from (58).

In the UK both historical data and sub-classification by ethnicity reflect similar patterns, as illustrated in Table 1.3. Myopia prevalence appears to have gradually risen over the last two centuries; Ware identified <1% prevalence in lower school children in 1831, in 1932 Sorsby examined children aged 4-14 years identifying a 4.0% prevalence, and in 2010 a 17.7% prevalence was reported in 12-13 year old children as

part of the Northern Ireland Childhood Errors of Refraction (NICER) study (8, 100, 101). There is a paucity of data in adolescent UK populations, with the majority focused on non-population based cohorts; in a study of refractive error in Aston University students, with a mean age of 19.6 years, myopia prevalence estimates of 50% for white and 53.4% for British Asian students were obtained (102).

Author/ Study Group	**Year**	**Myopia Prevalence (%)**	**Age Range (years)**	**N**	**Method & cut-off**
Ware **(103)**	1813	<1%	Lower school	1,300	Not known
Thompson **(104)**	1919	18.8	High school	3,249	Clinic sample
Mcllroy and Hamilton **(105)**	1932	21.6	6-14	1,702	Non-cycloplegic
Sorsby **(106)**	1932	21.6	6-14		Cycloplegic
McNeil **(107)**	1955	21.2	5-15	1,066	Clinic Sample
Rudnicka / CHASE Study **(108)**	2010	3.4	10-11	1,179	Non-cycloplegic ≤ -0.5D
O'Donoghue / NICER Study **(101)**	2010	2.8 17.7	6-7 12-13	1,053	Cycloplegic ≤ -0.5D
Logan / Aston Eye Study **(109)**	2011	9.4 29.4	6-7 12-13	327	Cycloplegic ≤ -0.5D

Table 1.3 Myopia prevalence in children of white European ethnicity in UK-based studies. CHASE = Child Heart and Health Study in England, NICER = Northern Ireland Childhood Errors of Refraction study. Adapted from (100)

Significant ethnic differences are also evident in myopia rates in children in the UK; in the Aston Eye study myopia prevalence in 6-7 year olds was 5.7% in white Europeans, and 10.8% in children of South Asian ethnicity (109). Similarly, at the age of 12-13 around 18.6% of white European children were myopic whereas the prevalence in South Asian children was 36.5% (109). Similarly in the Child Heart and Health Study in England (CHASE) study myopia prevalence was 3.4% in white European children, 10.0% in black African Caribbean children, and up to 25.2% in children of South Asian

ethnicity (108). There is evidence for increasing myopia prevalence in UK children; in the NICER study conducted between 2006-2008 the proportion of myopes aged 12-13 years was 14.6%, far higher than that reported for children aged 10-15 years in historical data from the UK in the 1960s (7.2%, p=0.01) (106, 110). Interestingly the authors also noted that in their white European population they had far higher rates of myopia at age 12-13 years (16.4%) compared to the 4.4% reported in the Australian Sydney Myopia Study (SMS) and follow-up Sydney Adolescent Vascular and Eye Study (SAVES) (111).

1.33 Projections and implications

The rising trends in myopia prevalence internationally (3, 50, 86, 87, 97) raise concerns for the future visual and financial burden from myopia. The National Eye Institute (NEI) project the numbers with myopia in the US will rise from ~30 million in 2010 to over 40 million in 2050 (64). The temporal trends in myopia from 2000 through to 2050 were estimated from a meta-analysis of prevalence rates and projection of future burden using change estimates over time derived from regression analysis (112). The current figure of 1406 million with myopia globally (22.9% of the world population) could increase to 4758 million (49.8% of the world population).

The refractive correction of ever increasing numbers of individuals, potentially half of the world's population, has significant financial implications. Current estimates of the financial cost of refractive correction for myopia in the US are $250m (£173m) per year (113). The worldwide market for glasses and contact lenses alone is expected to grow from $81 billion in 2011 to $130 billion in 2018 (114). There is also concern that even when appropriate refractive correction is provided, the rates of associated visual impairment associated with pathological myopic complications will increase. Myopic maculopathy, currently untreatable and attributable to between 12-14% of visual impairment in working age adults (56, 57), is likely to become more a bigger problem with rising levels of myopia. This is a particular concern for those with high myopia (48), and similarly projected to rise from affecting around 163 million people globally (2.7% of the world population) to 938 million people (9.8% of the world population).

1.4 Environmental factors for Myopia

Myopia is a highly complex trait influenced by both genetic and environmental risk factors (115). I will use the term 'environmental' factors to describe any lifestyle

factors (e.g. time spent outdoors), personal attributes (e.g. intelligence), personal achievements (e.g., education), and sociological factors (e.g., urbanisation). There are potential flaws in this approach as some of these factors may not be considered traditional, and modifiable, environmental factors (e.g. intelligence) and many are subject to genetic influence. However, for the purposes of my research, where I compare analyses to twin modeling in which trait variance is decomposed into the binary division of genes and environment, I will hereafter refer to all factors that are not gene loci or twin model estimates of genetic risk as environmental.

Previous research has identified a number of key environmental associations that I will consider in turn, and it is widely accepted that it is a combination of these changing environmental exposures that are the cause of the recent global trends in myopia prevalence. In view of my research I will limit my consideration of environmental factors to those affecting risk of myopia onset rather than myopia progression.

1.41 Education

Education is one of the most replicated and influential risk factors for myopia (4, 7, 9, 116-118); both progression onto higher education and educational attainment are strongly associated with myopia. The first to identify a higher prevalence amongst university students in 1813 was James Ware, a St Thomas' eye surgeon and Fellow of the Royal Society, who reported that "In the Guards short-sightedness among the privates is scarcely known; and not more than half-a-dozen recruits are said to have been rejected for this imperfection in the course of twenty years. In the universities, on the contrary, the numbers are so considerable, that in one of the colleges in Oxford, it is said that of one hundred and twenty-seven persons, so many as thirty-two have used either a hand-glass or spectacles" (103). This association was observed repeatedly during the 19th and 20th centuries in the UK, the US, Europe and Asia [Table 1.5]. Estimates of myopia prevalence in university students by the early part of the 20th century ranged from 15.0 to 35.0% (100).

Year	Prevalence (%)	Location	Investigator
1871	43.0	Germany	Erismann
1875	40.0	Germany	Pfluger
1876	76.0	Switzerland	Emmert
1877	49.0	England	Kotelmann
1877	35.0	Harvard, USA	Derby
1879	81.0	Germany	Seggel
1880	20.0	England	Smith
1881	53.0	Germany	Cohn
1881	30.0	Germany	Collard
1882	32.4	Denmark	Tscherning
1882	47.2	Amherst (upper division), USA	Derby
1883	35.0	Germany	Durr
1884	31.0	Germany	Van Anrooy
1885	52.5	Composite of 6 European and 2 USA studies	Randall
1887	28.5	Brooklyn Polytechnic, USA	Agnew
1887	40.0	New York College, USA	Agnew
1907	50.0	Germany	Fleischer
1911	15.0	University of California, USA	Burnett
1920	55.0	China	Li and Rush
1924	20.0	England	Clarke
1932	12.0	Japan	Tamura
1933	35.0	India	Banerjeee
1935	35.0	Washington, USA	Hall
1935	24.0	Switzerland	Francesschetti
1951	32.0	England	Parnell
1968	44.0	Harvard (Business and Law Schools), USA	Dumphy
1984	75.0	Optometry students, USA	Septon
1985	81.0	Optometry students, USA	Schell

Table 1.4 Myopia prevalence in university students 1871-1985, adapted from (100)

In more recent publications the association with higher education / educational level / years of schooling has been widely replicated. There is recent evidence for an effect of educational level in populations of African (73, 119), North African and West Asian (120), European (20, 85, 121-125), Northern East Asian (126), and Southeast / East Asian descent (66, 127-129). A successive increase in myopia prevalence across nine levels of education was observed in military conscripts (aged 15 to 25 years) in Singapore between 1987-1992; from ~15% myopia prevalence in those with no formal education to ~65% in those who had successfully completed 3-5 years of university education (127). In the German Gutenberg Health Study (GHS, participants aged 35 to

74 years), published in 2014, a similar dose-response to years of education was identified; those who graduated after 13 years of education were more myopic (median SE -0.5D) than those who graduated after 10 years of education (median SE -0.2D), than those who graduated after 9 years of education (median SE 0.3D), and than those who never finished secondary school (median SE 0.2D, $p<0.001$) (117). Similarly, the myopia prevalence in university graduates, graduates of secondary and primary vocational schools, and those without any professional training was 53%, 34.8%, 34.7%, and 23.9% respectively ($p<0.001$).

Educational achievement, as determined by higher scores in academic tests, is also repeatedly associated with myopia (120-122, 130-133). Myopia and educational attainment were correlated in the GEnes in Myopia (GEM) study ($r= - 0.21$, $p<0.01$), where up to 4.4% of variance in refraction could be explained by educational attainment (5). The authors additionally performed bivariate twin modeling which confirmed that genetics explain a large component of both educational attainment and refractive error variance, and indicated that part of that genetic risk may be shared between the two traits (116). Following this, the interaction between recently identified genetic variants for refractive error and educational level was examined in a European cohort; individuals at high genetic risk in combination with university-level education had a very high odds of myopia (OR 51.3) compared to those with a high genetic risk but only primary level education (OR 7.2) (134). In the Gutenberg Study the cumulative effect of known genetic variants for myopia was examined in a multivariate model. Whilst the genetic variants for myopia to date have a small effect on myopia risk, it is interesting to note that the effect of education remained both significant and of similar effect size when adjusted for genetic risk; the beta coefficient in a mixed linear model for spherical equivalent was -0.49 ($p<0.001$) with a university degree and -0.54 ($p<0.001$) following incorporation of genetic risk into the model (117). Conversely the cumulative effect of known genetic variants linked with educational attainment was examined in a Mendelian randomisation study where the direct and indirect effects of education and education-related genetic variants were examined (135). The authors noted that their estimate of the effect on education on myopia using genetic predisposition as an instrumental variable (-0.92 D per approximate 2 years in education, $p=0.001$) was higher than an observational estimate (-0.25 D per approximate 2 years in education), thereby meaning observational studies may underestimate the true effect of education, and that educational attainment has a

causal role on myopia risk. The CREAM consortium has examined gene-environment-wide associations across multiple European and Asian populations; three genome-wide significant loci displayed a significant interaction with education (*AREG, GABRR1, PDE10A*) in Asian populations, with interactions less evident in European populations (136).

There appears to be an association between myopia and modern education. Globally higher educational achievement is correlated with the prevalence of myopia (118), albeit with exceptions in low income countries, such as the South Pacific Islands of Vanuatu (137), and in rural areas, for example in rural China (138) and Taiwan (139). It has been argued that modern education systems may in part be the culprit of the recent epidemic of myopia, something I have examined within a European population in Chapter 3.3.

The educational level achieved by high myopes in adult and paediatric populations was examined in a meta-analysis published in 2016; the authors examined cohorts largely from China but also other Asian countries (140). They observed that whilst educational level achieved was higher in the children with high myopia, in adults the educational level did not differ between the highly myopic group and the comparative non-highly myopic group. They concluded that two forms of high myopia may exist – 'new' high myopia in today's school children and 'old' high myopia in today's elderly generation, also referred to as genetic given the proposed lack of environmental pressures in this generation. The authors went on to conclude that education-related parameters might be a cause of 'new' high myopia. It has also been proposed that educational styles and the interaction of education with other lifestyle factors (eg. how leisure time is spent) may have a significant effect on myopia risk; in populations of European ethnicity it has been noted that myopia prevalence is higher in European and North American countries compared to Australia (7, 110). There is also evidence that educational methods may be influential; for example high myopia rates were noted in Taiwan, Singapore and other countries where engagement in after-school intensive tutorials was high. Whereas in countries with comparable, top quartile educational outcomes (as measured by the Program in Secondary Assessment (PISA) reports in 2009), such as Finland and Australia, where there was minimal engagement in after-school intensive tutorials, myopia rates were lower (118).

1.42 Intelligence

Closely related to education, intelligence has also been independently associated with myopia risk. Higher cognitive ability, measured using various techniques but furthermore referred to as intelligence or intelligence quotient (IQ), has been associated with higher rates of myopia. This has been observed internationally including in Israel, Denmark, United States, New Zealand, Singapore, United Kingdom and Iran (2, 120, 121, 130, 141-145). There has been consistent evidence of a link between myopia and intelligence since it was first observed by Cohn in 1883 (146), this association is inclusive of child and adult samples, and independent of years of education completed (120, 147). An odds ratio for myopia of 2.4 for those in the highest IQ quartile was identified in the Singapore Cohort Study Of the Risk Factors for Myopia (SCORM) study, and an odds ratio of 1.35 for those in the highest IQ tertile in the British Avon Longitudinal Study of Parents and Children (ALSPAC) study (2, 148).

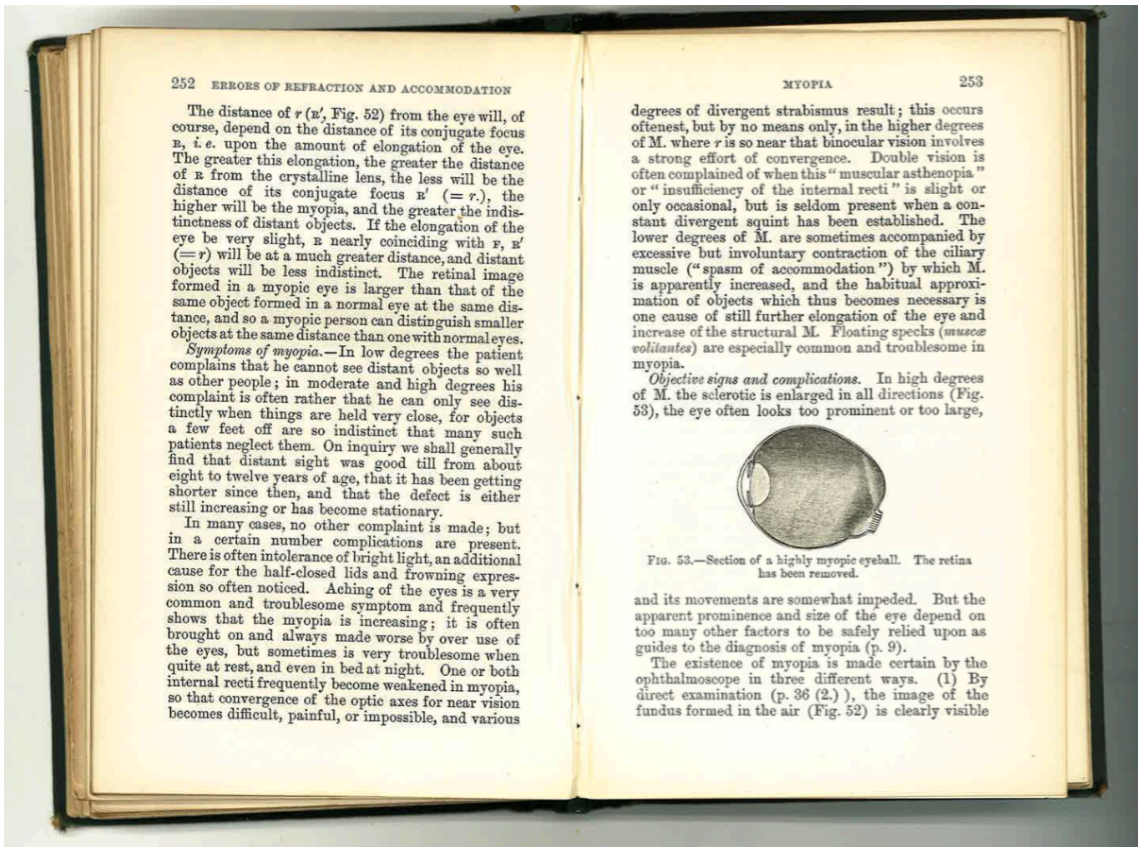
There are various hypotheses as to the underlying causal pathway. It has been hypothesised that myopic children, with their heavy glasses, are less likely to play outdoor sports and are more likely to spend time on their school studies, therefore increasing the chance that they reach a high level of intelligence. This theory is however considered unlikely, primarily due to the lack of evidence that a large effect on IQ can be achieved by studying alone (149). The other popular theory is that highly intelligent children increase their probability of becoming myopic by spending more time on near-work activities such as reading (150-152). However the association between near work and myopia is not robust (153, 154), and refraction in pre-school children can fairly accurately predict those who will become myopic, prior to periods of intense near-work at school (11, 31).

A number of researchers have therefore proposed an alternate theory that genetic factors may be important in determining the risk of both myopia and higher intelligence, particularly considering their high heritability. Early proponents for this theory include Karlsson who identified an association between myopia and IQ where the measurement of high IQ was obtained prior to the development of myopia (155). This view was echoed by Benbow (156) and Cohn (157) who identified higher rates of myopia in more intelligent children, with less myopia in their less gifted siblings. Karlsson went on to propose a theory for a single myopia gene influencing brain development with ‘evolutionary advantages for urbanised living’ (158). This

hypothesis has later been endorsed by Miller (159) and Cohn (157). This is a topic I explore further in Chapter 5.4.

1.43 Near work

The association between near work and myopia has been appreciated since the 17th century; Kepler stated that "Those who do much close work in their youth become myopic" in 1611 (160). Another St Thomas' Ophthalmologist, Edward Nettleship, wrote in his 'Student's Guide to Diseases of the Eye' published in 1879 (Figure 1.7) that myopia "is often hereditary, several near relatives often being affected. But in most cases it is acquired by the prolonged use of the eyes in looking at objects held at a very short distance" (161). Nettleship goes on to explain that this brings on a "strain on the internal recti counterbalanced by a corresponding tension on the external recti … act(ing) by slightly bulging out the unprotected posterior pole of the sclerotic (sclera)." Whilst the latter theory is now considered unlikely, the teaching that genetics and near work are important in myopia risk is still relevant.



252 ERRORS OF REFRACTION AND ACCOMMODATION

The distance of r (R′, Fig. 52) from the eye will, of course, depend on the distance of its conjugate focus R, *i. e.* upon the amount of elongation of the eye. The greater this elongation, the greater the distance of R from the crystalline lens, the less will be the distance of its conjugate focus R′ ($= r.$), the higher will be the myopia, and the greater the indistinctness of distant objects. If the elongation of the eye be very slight, R nearly coinciding with F, R′ ($= r$) will be at a much greater distance, and distant objects will be less indistinct. The retinal image formed in a myopic eye is larger than that of the same object formed in a normal eye at the same distance, and so a myopic person can distinguish smaller objects at the same distance than one with normal eyes.

Symptoms of myopia.—In low degrees the patient complains that he cannot see distant objects so well as other people; in moderate and high degrees his complaint is often rather that he can only see distinctly when things are held very close, for objects a few feet off are so indistinct that many such patients neglect them. On inquiry we shall generally find that distant sight was good till from about eight to twelve years of age, that it has been getting shorter since then, and that the defect is either still increasing or has become stationary.

In many cases, no other complaint is made; but in a certain number complications are present. There is often intolerance of bright light, an additional cause for the half-closed lids and frowning expression so often noticed. Aching of the eyes is a very common and troublesome symptom and frequently shows that the myopia is increasing; it is often brought on and always made worse by over use of the eyes, but sometimes is very troublesome when quite at rest, and even in bed at night. One or both internal recti frequently become weakened in myopia, so that convergence of the optic axes for near vision becomes difficult, painful, or impossible, and various

MYOPIA 253

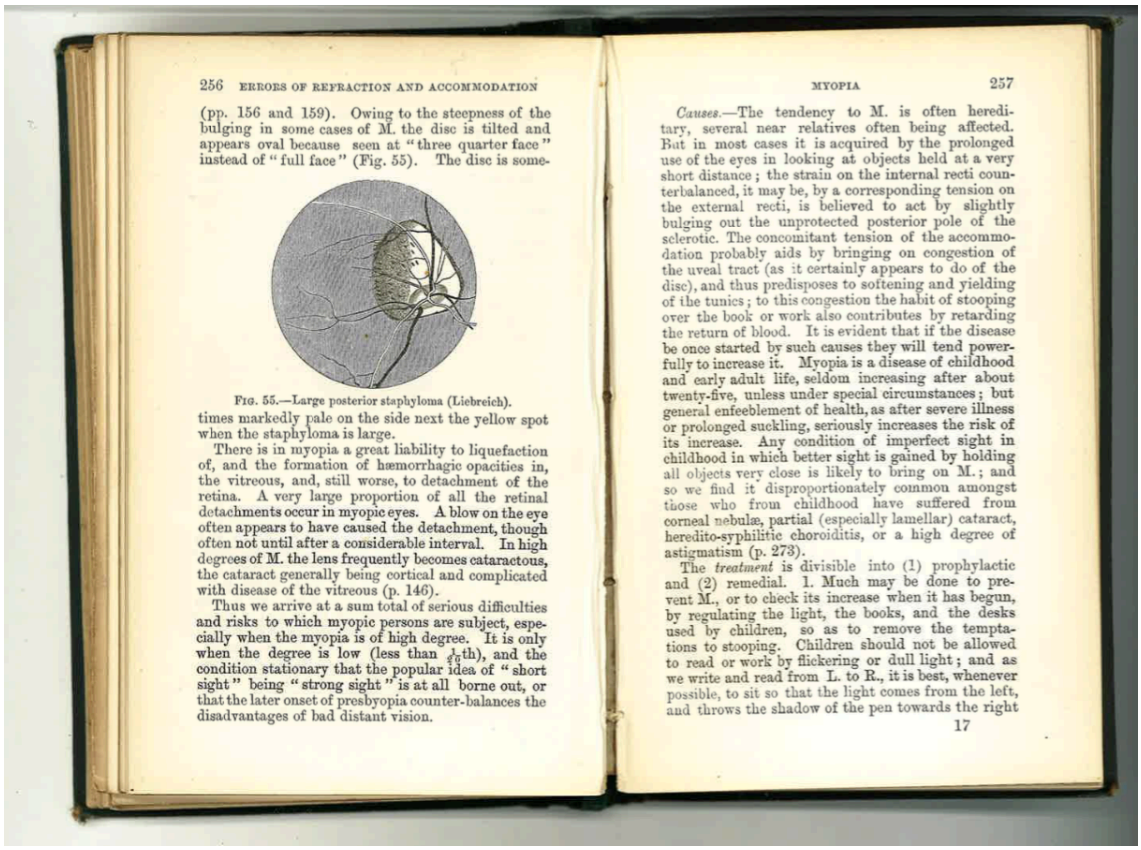
degrees of divergent strabismus result; this occurs oftenest, but by no means only, in the higher degrees of M. where r is so near that binocular vision involves a strong effort of convergence. Double vision is often complained of when this "muscular asthenopia" or "insufficiency of the internal recti" is slight or only occasional, but is seldom present when a constant divergent squint has been established. The lower degrees of M. are sometimes accompanied by excessive but involuntary contraction of the ciliary muscle ("spasm of accommodation") by which M. is apparently increased, and the habitual approximation of objects which thus becomes necessary is one cause of still further elongation of the eye and increase of the structural M. Floating specks (*muscæ volitantes*) are especially common and troublesome in myopia.

Objective signs and complications. In high degrees of M. the sclerotic is enlarged in all directions (Fig. 53), the eye often looks too prominent or too large,

FIG. 53.—Section of a highly myopic eyeball. The retina has been removed.

and its movements are somewhat impeded. But the apparent prominence and size of the eye depend on too many other factors to be safely relied upon as guides to the diagnosis of myopia (p. 9).

The existence of myopia is made certain by the ophthalmoscope in three different ways. (1) By direct examination (p. 36 (2.)), the image of the fundus formed in the air (Fig. 52) is clearly visible



256 ERRORS OF REFRACTION AND ACCOMMODATION

(pp. 156 and 159). Owing to the steepness of the bulging in some cases of M. the disc is tilted and appears oval because seen at "three quarter face" instead of "full face" (Fig. 55). The disc is some-

FIG. 55.—Large posterior staphyloma (Liebreich).

times markedly pale on the side next the yellow spot when the staphyloma is large.

There is in myopia a great liability to liquefaction of, and the formation of hæmorrhagic opacities in, the vitreous, and, still worse, to detachment of the retina. A very large proportion of all the retinal detachments occur in myopic eyes. A blow on the eye often appears to have caused the detachment, though often not until after a considerable interval. In high degrees of M. the lens frequently becomes cataractous, the cataract generally being cortical and complicated with disease of the vitreous (p. 146).

Thus we arrive at a sum total of serious difficulties and risks to which myopic persons are subject, especially when the myopia is of high degree. It is only when the degree is low (less than $\frac{1}{6}$th), and the condition stationary that the popular idea of "short sight" being "strong sight" is at all borne out, or that the later onset of presbyopia counter-balances the disadvantages of bad distant vision.

MYOPIA 257

Causes.—The tendency to M. is often hereditary, several near relatives often being affected. But in most cases it is acquired by the prolonged use of the eyes in looking at objects held at a very short distance; the strain on the internal recti counterbalanced, it may be, by a corresponding tension on the external recti, is believed to act by slightly bulging out the unprotected posterior pole of the sclerotic. The concomitant tension of the accommodation probably aids by bringing on congestion of the uveal tract (as it certainly appears to do of the disc), and thus predisposes to softening and yielding of the tunics; to this congestion the habit of stooping over the book or work also contributes by retarding the return of blood. It is evident that if the disease be once started by such causes they will tend powerfully to increase it. Myopia is a disease of childhood and early adult life, seldom increasing after about twenty-five, unless under special circumstances; but general enfeeblement of health, as after severe illness or prolonged suckling, seriously increases the risk of its increase. Any condition of imperfect sight in childhood in which better sight is gained by holding all objects very close is likely to bring on M.; and so we find it disproportionately common amongst those who from childhood have suffered from corneal nebulæ, partial (especially lamellar) cataract, heredito-syphilitic choroiditis, or a high degree of astigmatism (p. 273).

The *treatment* is divisible into (1) prophylactic and (2) remedial. 1. Much may be done to prevent M., or to check its increase when it has begun, by regulating the light, the books, and the desks used by children, so as to remove the temptations to stooping. Children should not be allowed to read or work by flickering or dull light; and as we write and read from L. to R., it is best, whenever possible, to sit so that the light comes from the left, and throws the shadow of the pen towards the right

17

Figure 1.8 Extracts from Edward Nettleship's 1879 'Student's Guide to the Diseases of the Eye'

In more recent literature many have found an association between various measures of near work and myopia; including self-reported prolonged periods spent reading (162, 163), quantifiable periods of multiple near work activities in combination measured in 'dioptre hours' (133), and children reporting that they read multiple books per week (150). A related variable is the 'liking' of reading, unless one argues that it represents a more complicated behavioural phenotype, and this too has been associated with myopia (2).

However, the association between myopia and near work is often weak or insignificant in epidemiological studies, inclusive of Asian, American and British populations (10, 132, 164-168). In the Sydney Myopia Study there was similarly a weak correlation between spherical equivalent and near work activities ($r \leq 0.2$) (169), but a stronger relationship between myopia and intense near work - a close reading distance (< 30 cm) and continuous reading (> 30 minutes) independently increased the odds of myopia in their sample. Historically, the dominant theory as to what causes myopia has been the combination of hyperopic defocus from accommodative lag and prolonged near work. However, some argue that this process has not been shown to be true clinically. In Mutti and Zadnik's 2009 article 'Has near work's star fallen?' they argue in infancy children have a good ability to accommodate and overcome their relative hyperopia with no correlation between measured defocus and refractive error change during emmetropisation, thus going against the theory that emmetropisation is a dose-dependent response to hyperopic defocus (69). There is conflicting evidence as to whether accommodative lag precedes myopia – elevated lag was reported two years before myopia onset by Gwiazda et al (170), whilst in the Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error (CLEERE) study accommodative lag was not elevated until a year after myopia onset (171). Subsequently evidence from the CLEERE study suggested that, although myopes definitely have increased levels of accommodative lag (172, 173), it does not appear to be a robust predictor for onset of myopia or highly progressive myopia (69). This conflicting evidence for the role of near work and accommodation in myopia risk is replicated in clinical interventions – whilst interventions that reduce accommodative load have some effect, ultimately myopia progression is not halted (174-177).

The association between myopia and education is traditionally thought to be mediated by prolonged periods of near work. However, a number of researchers have highlighted that it may be underlying intelligence rather than time on near work that underlies the association with myopia (132). In the Singapore Cohort Study of the Risk Factors for Myopia (SCORM) study parental myopia and a higher IQ increased the risk of myopia onset but near work did not (165). Others have also theorised that the association with near work may be mediated by a shared genetic tendency for myopia and enjoyment of reading in the parents and offspring, or that parental myopia may confer a home environment where reading is encouraged over outdoor activities (178). In twin studies, the contribution of shared environmental effects on refractive error variance has been estimated at 7% (179). In epidemiological studies, without the use of genotypic data, an interaction between parental myopia and near work has been variably detected (165, 166, 180).

This topic is widely discussed outside of the ophthalmic community and something I hope to explore further; if proxy measures of near work activity are not robustly associated with myopia why is that so many myopes are avid readers? In the writer Dame Penelope Lively's recent memoir, highly myopic since early childhood and now a sufferer of myopic macular degeneration, she notes: "One-sixth of the world's population is myopic, but amongst readers the proportion is much higher — about a quarter. Habitual readers, that is — those who spend much time reading. Which raises the intriguing question of whether we book-addicted are thus because of some genetic conditioning or whether we have wrecked our eyesight through our addiction" (181).

1.44 Time outdoors

The protective effect of time outdoors on myopia risk has been identified in a number of child and adolescent cohorts over the last two decades (182). It appears that children who spend more time outdoors are less likely to have or develop myopia and this finding has been replicated in children from different climates including Europe, USA, Singapore and Australia (133, 166, 167, 183-185), although notably a lack of significant association with myopia progression has been identified in the CLEERE, Anyang, and early-onset myopia in the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) studies (164, 168, 186). When identified the association remains when adjusted for potential confounding factors, such as near work, which may be heightened in children who avoid spending time outside, or parental myopia, which

could be hypothesised to contribute to a shared family environment of avoidance of outdoor activities (162, 166). A meta-analysis of time spent outdoors and myopia in seven cross-sectional studies concluded a 2% reduced odds of myopia per additional hour of time spent outdoors per week, after adjustment for covariates (187). This provides an attractive therapeutic option for reducing the development of myopia, and potentially also the rate of myopia progression.

Reports in the literature of therapeutic interventions that harness the protective effect of time outdoors are emerging with positive results, such as encouraging children to spend recess periods outside, structured weekend outdoor activities and pedometers to encourage increased walking (188-190). In the Guangzhou Outdoor Activity Longitudinal (GOAL) study schools were randomly allocated to an intervention aimed to reduce myopia – an additional 45 minute outdoor activity class at the end of each day (191). Preliminary results suggested that spending more time outdoors reduces the number of incident cases of myopia (the primary outcome measure). More recently in a randomised clinical trial among 6 year olds in China an additional 40-minute class of outdoor activities reduced the incidence of myopia over a three-year period by nearly 10% (189). This therapeutic intervention has been pushed even further in one school in Yang Jiang, Guangdong province where all the walls have been made of glass, thus bathing the classroom in natural daylight (64). Results from this therapeutic intervention are yet to be reported. It appears that time outdoors is better at reducing myopia onset than progression - only a few studies show any effect of the refractive error of those already myopic (188). Reasons underlying this observation could be that once emmetropisation has failed and the child is myopic, alterations to light levels or potentially other environmental factors can have little effect on the 'uncontrolled' trajectory of ocular axial growth.

Some have suggested that time outdoors may reduce the time a child spends on near work activities, a potential risk factor for myopia as discussed above. However Rose et al, in the Sydney Myopia Study (SMS), found that even if high levels of near work were reported in combination with long periods of time outside the odds of myopia still remained relatively low (OR ~0.5), whereas those who combined high periods of near work with low levels of time outside had a high risk of myopia (OR ~2.6), illustrated in Figure 1.7 (185). Similarly in the Sydney Adolescent Vascular and Eye Study (SAVES), a 5-6 year longitudinal follow-up of SMS, near work only showed significant association

with incident myopia in the younger cohort (aged 6 years at baseline), whilst time spent outdoors was negatively associated with incipient myopia in both age cohorts (6 and 12 years old at baseline) (162).

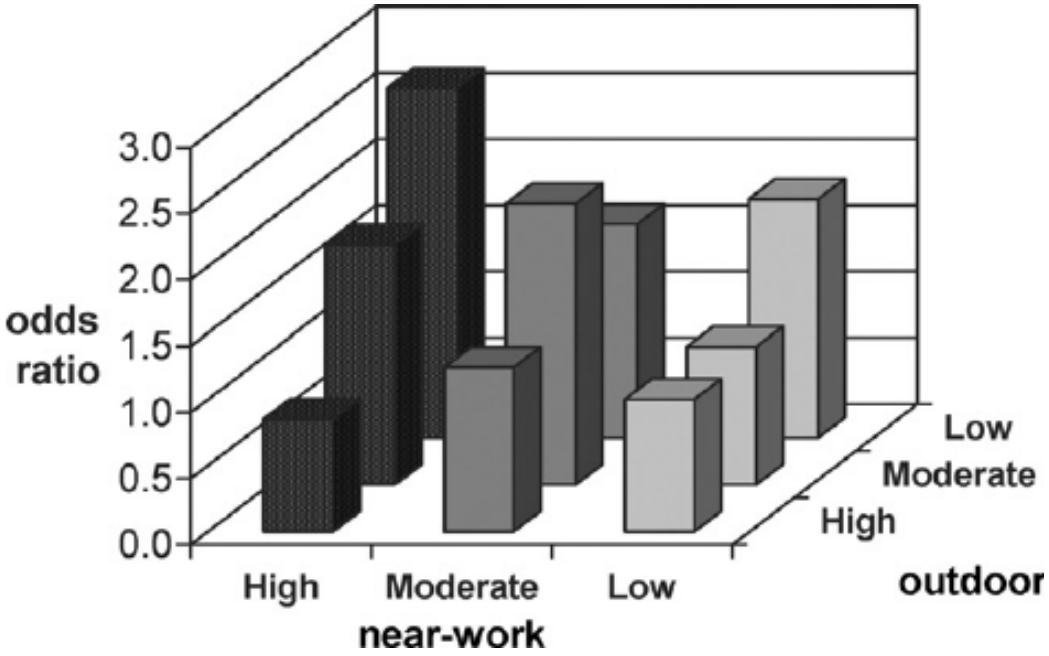


Figure 1.9 Multivariable-adjusted odds ratios (adjusted for gender, ethnicity, parental myopia, parental employment, and education) for myopia by reported average daily hours spent on near work versus outdoor activities in 12 year-olds. Reproduced with permission from (185)

It is not clear which aspect of spending time outdoors mediates the protective effect. Initial research suggested an association between myopia and sports/outdoor activities (153, 166). The important question of whether being outside or being engaged in physical activity reduced the chance of myopia was answered by subsequent research where the two factors were considered separately; the authors concluded incident myopia was primarily due to time outdoors rather than physical activity or indoor sports (185, 192). In the Avon Longitudinal Study of Parents and Children (ALSPAC) children who spent less time outdoors were 40% more likely to become myopic, whilst low physical activity only increased the risk of myopia by 10%. Through spending more time outdoors, one is exposed to a number of factors - a brighter light intensity, a different UV spectrum to that emitted by artificial lighting, and, generally, a longer focal distance.

Some argue that distant focus is highly relevant as peripheral retinal blur, now known to be a major stimulus to eye growth, is reduced when a distant object is viewed (48). Therefore distant focus could provide a 'stop signal' to the emmetropisation process and eye growth (193). However, there is a wealth of convincing literature surrounding the protective effect of light. Increased light intensity, both sunlight and intense laboratory lights, appears to retard the development of form deprivation models of myopia in chicks (194), and this is echoed in human studies. Epidemiology studies suggest populations living near the Arctic Circle, including a cohort of Finnish army conscripts, where the sun never rises during the winter months have higher rates of myopia (195, 196). Similarly a high rate of myopia has been identified in submariners who spend long periods of time without seeing natural daylight, although this environment is additionally subject to limited distant focal viewing (197, 198).

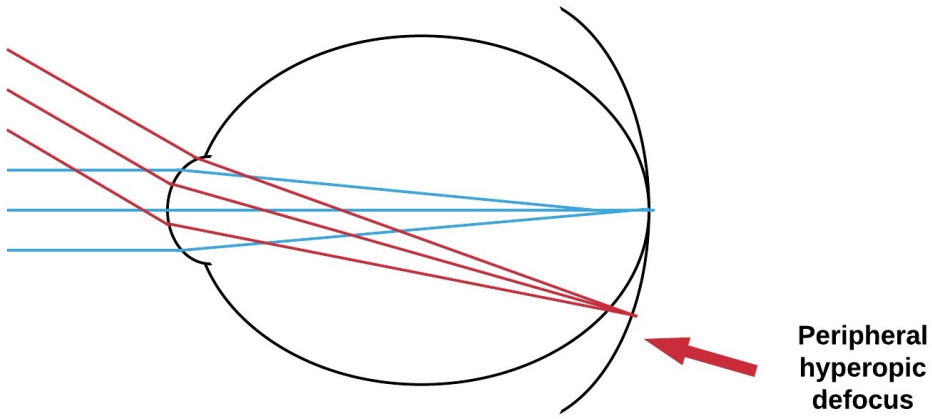


Figure 1.10 Peripheral hyperopic defocus. The elongated, prolate shape of a myopic eye with light in focus on the central region of the retina and relative hyperopic defocus in the periphery, inducing peripheral retinal blur and potentially axial elongation (199, 200).

Differences in the illuminance and the wavelength spectrum of light differ markedly between the indoor and outdoor environments experienced by humans. Artificial lighting is typically less than 1000 lux, whereas direct sunlight on a clear day outdoors is approximately 130,000 lux or 15,000 lux in the shade (201). Sunlight contains a broad spectrum of wavelengths, including rays in the UV range; indoor lighting contains a more limited range of the spectrum. It is estimated the annual exposure of

UVR from artificial lighting is equivalent to just one week of outdoor activity in a sunny destination (202). Indirect markers of ocular sun exposure (in particular UVR) have also been associated with myopia; in the Australian Raine cohort and Norfolk Island study there was an inverse association between myopia and UV conjunctival autofluorescence, a sign of preclinical sun damage on the conjunctiva (187, 203). The authors highlighted that the protective effect of an indirect measure of UV exposure was greater than that identified through a questionnaire of time spent outdoors. One study has looked specifically at UV exposure and myopia (204), and found daily exposure differed significantly between emmetropes, stable myopes and progressive myopes. I explore this issue further in Chapter 4.3.

Proposed mechanisms by which the protective effect of outdoor light on myopia is mediated include fluctuations in serum vitamin D and the activation of dopaminergic pathways (205). Dopamine, released from dopaminergic retinal amacrine cells, which are stimulated by both light and activated by intrinsically photoresponsive retinal ganglion cells (201, 206), is known to influence axial growth of the eye (207, 208). In animal studies, form deprivation and lens induced myopia are associated with lower levels of dopamine metabolites whilst dopamine antagonists and agonists, in particular those affecting the D2 receptor, have been observed to induce and reduce myopia development respectively (201). Another proposed mechanism is via increased serum levels of vitamin D, which occur after exposure to sunlight. An association between myopia and increased serum levels of vitamin D has been identified a number of epidemiological studies, with variable adjustment for potentially confounding factors such as time spent outdoors and sunlight exposure. Genetic polymorphisms in vitamin D pathway genes have been associated with myopia, and also with other ocular diseases such as age-related macular degeneration (209-212). However when time spent outdoors is considered in conjunction with vitamin D levels and myopia it appears that, although there is an association between vitamin D and myopia, the protective effect of time outdoors on myopia is not mediated solely by vitamin D (213).

1.45 Urbanisation

Living in a more urban rather than rural environment was most elegantly illustrated when the Inuit tribes of North America were observed to develop increasing rates of myopia as they moved off the ice plains into urban settlements (214). This finding has been replicated in numerous other cohorts, and is interestingly also observed when

individuals of similar genetic ethnicity reside in different environments (7, 50, 93, 138, 215-220) although with one exception (10).

Reasons underling this association have been postulated as differences in educational levels and socioeconomic status in urban versus rural populations, both risk factors for myopia, or differences in time spent outdoors and distant focus when living in a crowded city. In Flitcroft's 2012 paper he suggests that the beneficial effects of time outdoors and the adverse effect of an urban environment are underlined by the three dimensional structure of the viewed environment and its impact on patterns of defocus across the retina (48). He presents evidence that the refraction of the eye must not be considered in one dimension, and that the off axis performance of the eye and eye shape have an effect on retinal image defocus; as such a standard office with documents on a desk and a computer screen present large amounts of peripheral hyperopic defocus from the three dimensional environment. He additionally argues that in a myope the eye is generally prolate shaped, therefore off-axis performance is very different to foveal performance.

1.46 Socioeconomic Status

Socioeconomic status (SES) has been associated with myopia with a higher socioeconomic status showing an inverse association with refractive error and a positive association with myopia risk (4, 9, 34, 88, 125, 126, 221, 222). Related measures of wealth such as deprivation scores and household income thresholds have shown less consistent association with myopia (10, 223).

1.47 Parental factors

A number of parental factors have a bearing on the risk of myopia, some of which may be in part be mediated through shared genetic factors. SES, generally defined by paternal employment, is associated with myopia. Some argue that given that SES modifies a child's intelligence, largely by environmental factors rather than genetic (224), the association pathway with myopia may be more complicated.

A number of maternal factors have been variably implicated in myopia risk; maternal age (9, 225, 226), smoking during pregnancy (9, 227), and maternal height (9). Some have suggested that the risk of myopia from an older maternal age may be mediated by a lower birth weight (228) as older mothers are more likely to give birth to babies of a

low birth weight (229), which itself has been variably linked to myopia risk (see below). Breastfeeding, beneficial for many aspects of life, has shown no significant association with myopia risk (9, 223, 226); in a meta-analysis by Rudnicka et al the adjusted myopia odds ratio (inferred from reduced unaided vision) showed no association with breastfeeding in three British cohorts (1946 birth cohort, 1958 birth cohort and 1970 birth cohort, n~40,000) (226).

1.48 Others

Anthropometric measures over the life course have shown variable associations with myopia. Low birth weight is associated with myopia in some studies but not in others (9, 223). The researchers of the SCORM study identified a positive association between birth length, head circumference and axial length, but no relationship with refractive error (230). A positive association between height and myopia has been reported (231), especially in high and early-onset myopia in the 1958 British Birth Cohort (9). In the Guangzhou twin study axial length was correlated with height (r=0.46), and an estimated 89% of this phenotypic sharing was due to shared genetic factors (232). A positive association between increasing weight and myopia has also been reported, particularly in females in the GEM study (233). The association between myopia and height, weight, BMI, and also age of menarche and diabetes has been postulated to be mediated by high glycaemic index diets, increasingly eaten in urban cultures, but clinical evidence for this is yet to be produced (7). Conversely a high BMI has been inversely associated with high and early-onset myopia in the 1958 British Birth Cohort (9), and many authors detect no association with height, weight or BMI (164). The complicated relationship between body stature growth trajectories during childhood and ocular growth and refractive error were reviewed in the ALSPAC study (234); the authors concluded that up to the age of 10 years, shared growth mechanisms controlling height, axial length and refractive error were evident. However the positive association between height and axial length only explained 1-5% of axial length variation, whilst the negative association between height and refractive error explained even less variation (<0.5% of refractive error variation) – thus they concluded that the effect of body stature on myopia development is minimal.

Other variables that have been associated with myopia include month or season of birth, and, related to this, the exposure natural light during the post-natal period (235-237). This variable is something I explore further in Chapter 4.25. Birth order has been

associated with myopia, with the first born at the highest risk of myopia and a dose-response relationship between sequential siblings and myopia risk evident in a study incorporating ALSPAC, SCORM, the Raine Eye Health Study (REHS) and Israeli Defense Force Pre-recruitment Candidates (238). However, a subsequent publication from the same researchers examining the same phenomenon but in the UK Biobank suggests that the effect is attenuated by education and that parents tend to invest more in the education of first born children (239). Also using the UK Biobank data, an association between childhood febrile illness and risk of myopia has been identified; this has been historically proposed (240) and this new research confirms the association but the underlying mechanism remains unclear (241).

Psychosocial associations with myopia have been reported. In the GEM study the relationship between myopia and five personality types were examined (Openness, Conscientiousness, Extroversion, Agreeableness and Neuroticism) (242). In adjusted models, Openness was the only significant personality trait that predicted myopia. Poor sleep, a risk factor for obesity, attention deficit disorder and poor academic performance amongst other things, has been proposed as a risk factor for myopia in China given the high prevalence of myopia and high prevalence of sleep disorders / reduced numbers of hours of sleep compared to the US and elsewhere. However, a Chinese study examining this issue found that although disordered sleep was common in their sample, there was no significant association with myopia risk (243).

1.5 Genetic aspects of myopia

Myopia is a complex trait influenced by genetic and environmental factors, but the contribution of genetic risk far outweighs the effect of environmental variables in explanation of trait variance. The dose-response association of one or more myopic parents and risk of myopia in their child is very well replicated (11, 27, 115, 133, 166). Formal heritability estimates for refractive error are very high (70-90%), as discussed below.

1.51 Twin Studies

Twin studies provide a unique opportunity to decompose a phenotype into the relative contribution of nature (genetics) and nuture (environment). They are based on the knowledge that monozygotic (MZ) twins share all of their genes, whereas dizygotic (DZ) twins share on average 50% of the same genes (the same as siblings). Galton is

regarded as the first scientist to identify the potential benefit of studying twins in 1874 (244). Since the early part of the 20th century numerous classical twin studies have been performed to compare concordance rates between identical (MZ) and non-identical (DZ) twins; for example an American educational psychologist identified greater similarity in intelligence tests in MZ compared to DZ twins in 1924 (245).

1.511 Uses, biases and assumptions of twin studies

The classical use of a twin study is to estimate heritability but they can also be used to examine disease prevalence/outcome in comparison to singletons, examine potential risk factors in discordant MZ twins, examine discordance for environmental factors in connection to disease rates in twins, linkage studies by using DZ twins as a special application of the sibling-pair design, and drug trials.

A number of potential areas of bias must be considered in twin studies;

1. Selection bias:
    a. Twin ascertainment bias – selection of twin pairs has historically been biased to those concordance for disease and must therefore be conscientiously avoided
    b. Volunteer bias – like all volunteer cohort studies, twin studies are prone to being predominantly female. There is also a propensity for MZ twins to volunteer more than DZ twins, despite the fact that at a population level DZ twins are more common
    c. Geographical bias – twins who live further apart are less likely to participate in a twin study
2. Information bias:

    This source of bias, common to all studies, can arise from measurement error of phenotypes and all covariates. In the case of heritability estimates, high levels of measurement error will result in lower estimates of the contribution of genetics to trait variance. Other examples are recall bias, which in the twin scenario may be heightened if the twins are concordant for that variable. To reduce this source of bias twins are ideally recruited for a study unaware of the subsequent phenotypes / conditions to be studied, using objective measures rather than subjective, self-reported measures, and interviewing the twins separately.
3. Confounding:

A variable can be incorrectly attributed to be very 'genetic' if there is a marked imbalance in a potential confounder between MZ and DZ twins; for example if the MZ twins are much older than the DZ twins then an age-related condition may be spuriously concluded to have a high heritability.

A number of assumptions are made of twin studies that must:

1. Equal environment assumption – this is the assumption that both MZ and DZ twins share their common environment to an equal extent (in utero and raised in the same family) (246).
2. Generalisability – whilst for most diseases twins are representative of the general population (247), it must be noted that twins have a lower birth weight than singletons and are born at approximately 37 weeks gestation, generally by caesarean section, with higher rates of neonatal morbidity and mortality.
3. Twin-twin interaction – it is assumed that the zygosity in no way influences behaviour or the phenotype being measured.
4. Assortative mating – like most genetic studies, twin studies assume the absence of assortative mating (ie. non-random mating).

1.512 Twin studies performed for refractive error and myopia

There have been numerous twin studies to estimate the heritability of refractive error. The earliest in 1922 provided general information on greater concordance for refractive error in 28 MZ twin pairs compared to 23 DZ twin pairs (248). More recent studies have used structural equation modelling to quantify the relative effect of genetics (additive (A) or dominant (D) genetic effects), the shared environment (C) and the unique environmental (E) on trait variance.

Recent estimates of refractive error heritability are summarised in Chapter 1.52, Figure 1. Twin estimates of heritability are consistently higher than those identified in family studies (249-251) and suggest genetic factors are important with heritability estimates of approximately 70-90% (67, 179, 252-257). In 506 British twin pairs from the TwinsUK cohort, univariant twin modeling suggested the variance of refractive error explained by genetic factors was 84-86% (252). This analysis identified the best-fitting model to explain the variance of spherical equivalent was the AE model, comprising of additive genetic factors (A) and the unique environmental factors (E). In a subsequent study on an extended TwinsUK cohort (2301 twin pairs) the ACE model

was used, to include the additional factor of the shared environment (C) as twin studies have a low power to detect shared environmental factors due to lack of age and generational differences, though these factors may be important (179). The heritability estimate was 0.77; the authors concluded that twin studies have more power to detect heritable effects than family studies.

1.52 Genome-wide association studies

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REVIEW

GWAS in myopia: insights into disease and implications for the clinic

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ABSTRACT

Myopia is the most common eye trait worldwide and the prevalence is increasing. It is known to be highly heritable; total genetic variation explains up to 70–80% of variance. In an attempt to better understand the genetic architecture of myopia, with an ultimate view to better predict genetic risk and develop targeted treatments, several genome-wide association studies have been performed in the last 6 years. In this review we focus on what a genome-wide association study involves, what studies have been performed in relation to myopia to date, and what they ultimately tell us about myopia variance and functional pathways leading to pathogenesis. The current limitations of genome-wide association studies are reviewed and potential means to improve our understanding of the genetic factors for myopia are described.

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Introduction

Myopia is already the commonest eye condition, and its prevalence is increasing across the world [1–4]. Refractive error is the term used to describe an error in the accurate focusing of light onto the retinal plane. Myopia, or near-sightedness, typically results from axial elongation of the eyeball, and this results in an image forming anterior to the retinal plane; hyperopia results when an image lies posterior to the retinal plane. Although myopia is strongly associated with a number of environmental factors, the most important risk factor in determining whether an individual develops the trait is a family history of myopia, suggesting a genetic predisposition. The heritability of a trait is an estimate of how much phenotypic variation in a population is due to genetic factors. The heritability of refractive error, using spherical equivalent as a quantitative trait, has been determined in a number of family and, more credibly, twin studies (Figure 1). These indicate the heritability of myopia is high at around 70% [5–15].

Myopia is a complex trait influenced by a complicated interplay of genetic and environmental factors. As with many complex traits, there is a distribution of refractive error in the population, meaning the risk of ordinary or 'simple' myopia developing is not determined by a classic Mendelian single-gene mode of inheritance; there are likely many genes, each contributing a small effect to overall myopia risk. This may not be true for very high, familial or syndrome-associated forms of myopia – in these cases, a rare dominantly inherited mutation may be important in an individual family, but not important in the overall population risk. Up until the era of genome-wide association studies (GWAS), identification of disease-associated genes relied on family studies (using linkage analysis) or candidate gene studies. In myopia, these were singularly unsuccessful and prior to 2009 there were no known myopia-associated genes, other than syndromes where myopia was a part of the phenotypic spectrum (e.g. Stickler's, Marfan syndromes). However, with the advent of GWAS, a number of genes for myopia have been identified, providing new insight into how myopia develops with implications for future research into how this increasingly common eye trait might be treated.

Genome-wide association studies

GWAS are approaches that allow a vast array of markers scattered across an individual's DNA or genome to be rapidly tested for association with a disease or trait. These 'markers' are variations in the base pair of nucleotides at specific points along the genome, commonly known as single-nucleotide polymorphisms (SNPs), and give an indication of what nearby genes may be associated with the trait.

In order for this analysis technique to be possible, all of the base pairs, namely adenine (A), guanine (G), thymine (T), or cytosine (C), forming the human DNA code had to be sequenced (i.e. read and mapped). The human genome project, completed in 2003, was a major international scientific collaboration that identified all of the base pairs and genes that make up the human genome, approximately 20,500 genes in total [16,17]. This has enabled researchers to have access to a detailed resource on the structure, function, and organization of the complete set of genes that make up the human species. However, to investigate the association between the human genome and disease, a 'map' of common patterns of genetic variation and inheritance was required, known as a 'haplotype map'. This was firstly provided by the HapMap project, completed in 2005 [18]; this international

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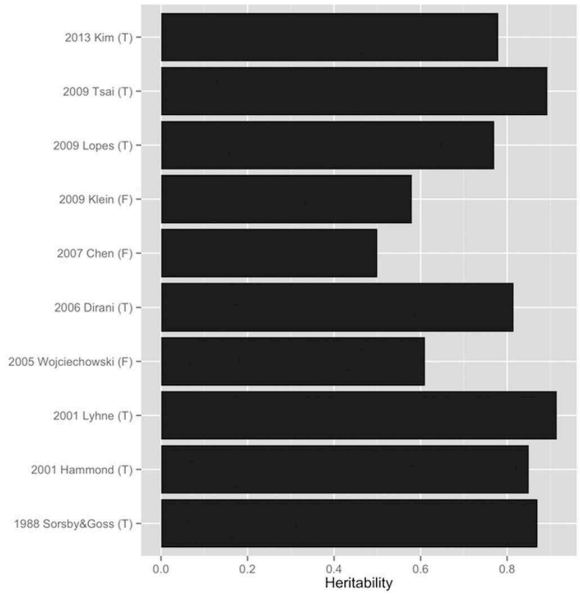

Figure 1. Heritability estimates for refractive error (Abbreviations: T = twin studies, F = family studies).

project compared the genetic sequences of individuals of African, Asian, and European ancestry. Subsequently, the 1000 Genome Project, which harnessed the increased speed, greater coverage, and reduced cost of next-generation sequencing, was launched. Released in 2012, this has provided the most detailed catalog of human genetic variation to date with sequencing of over 1000 participants internationally [19]. These maps of common inheritance patterns allow identification of what base pair is commonly at one position in the genome of a certain ethnic population, the 'common' allele, and what base pair tends not be at that position, the 'minor' allele. SNPs are generally termed a common polymorphism when the frequency of the minor allele, in a specific population, is greater than 1%.

GWAS rely upon the assumption that common complex traits are caused by common genetic variations in the population (the 'common disease common variant' hypothesis). Therefore, in a GWAS, the association between a trait and common genetic variants in the form of SNPs is examined. SNPs are not disease-causing mutations as found in classical genetic studies of rare Mendelian diseases, and they rarely alter protein structure or function, but instead they may relate to regulation of genes or alterations in gene expression. In GWAS, SNPs are used as markers and indicate nearby genes or biological pathways that may be involved, allowing researchers to focus in on specific parts of the genome.

To perform a GWAS for a disease, an individual must be genotyped or sequenced; in large-scale genetic studies, this is generally undertaken with the use of high-throughput genotyping arrays or chips. These provide an output of somewhere between 500,000 and 2,500,000 SNPs for that individual, but obviously do not include all the common genetic variants (given there are around 3 billion base pairs in the human genome). The missing data are, therefore, imputed using reference haplotypes, either the HapMap or 1000 Genome data. Associations between these genetic variations, following extensive data cleaning (quality control), and disease status are examined in regression models either as a quantitative trait (e.g. refractive error measured by spherical equivalent) or as a categorical case–control trait (e.g. 'myopia' or 'no myopia'). The output from such analyses is a list of associated SNPs with an indication of the strength of effect on myopia risk (the beta coefficient) and the confidence of the association (*p*-value). Significance thresholds are set at less than $p \leq 5 \times 10^{-8}$ to reduce the possibility of false-positive associations, which may occur as a result of correlation between SNPs and the high number of statistical tests involved. This means large studies of many thousands of individuals are required to identify statistically significant associations. Results are generally portrayed graphically as a Manhattan plot, which plots all the SNPs by chromosome position as a function of their association *p*-value; this plot resembles the Manhattan skyline with different SNPs reaching higher than others, like skyscrapers, in accordance with variations in significance. Results of putative genetic associations for a trait ('discovery stage') must then be verified through replication of associated variants in independent population samples or through experiments that can examine the functional implications of the affected gene.

The first GWAS was performed in 2005, and since then there has been an exponential rise in the number of studies (Figure 2), reflecting the large reduction in time and cost of undertaking these types of analysis.

GWAS have now been successfully performed on a range of ophthalmic diseases [21,22]. The earliest and arguably the most 'successful' GWAS to date has been within the ophthalmic field; the discovery of the association of CFH with age-related macular degeneration (AMD) was reported in three independent cohorts in 2005 [23–25], one of which was a GWAS, and has since been replicated in dozens of studies across the world. Subsequent meta-analysis involving large sample sizes (>17,100 cases and >60,000 controls) has identified 19 loci for AMD explaining 10–30% of the variance [26], which has an estimated heritability of 45–70%. These genetic associations explain a relatively high proportion of AMD variance, which disappointingly has proved to be fairly unusual in subsequent GWAS for other traits. Although GWAS have identified many variants for many diseases, relatively small effects on disease risk are conferred for the majority of variants and only a small proportion of familial clustering or heritability is explained. This issue of 'missing heritability' is a recurrent issue in GWAS and has prompted researchers to explore additional approaches to examine the genetic architecture of common complex diseases [27].

GWAS in myopia

Refractive error and myopia have been examined using the full range of genetic methodologies. This initially included genome-wide linkage studies in related individuals, which have identified at least 17 loci, and candidate gene association studies, which were rarely replicated [28–30]. The first GWAS

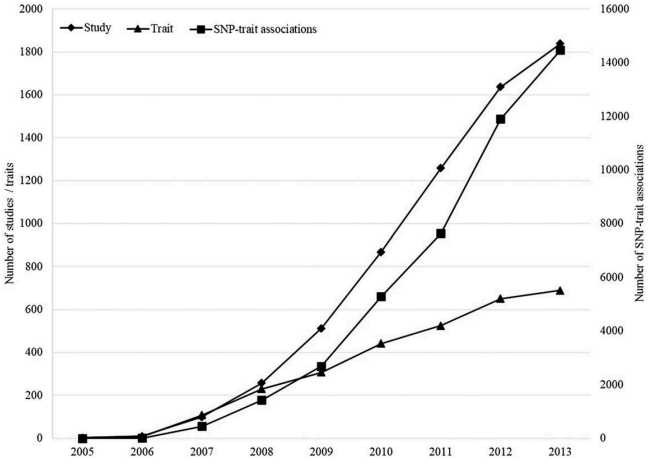

Figure 2. Studies, traits and SNP-trait associations from 2005 to 2013 reveal the growth in genome-wide association studies. (Reproduced from Welter et al. [20] by permission of Oxford University Press).

to examine myopia was performed in 2009 on a cohort with high, pathological myopia; subsequent studies have either been performed on myopia case–control cohorts, largely from East Asia where the prevalence of myopia and high myopia is greater, or on cohorts with refractive error measured as a quantitative trait. The GWAS catalog database detailing all published GWAS for myopia, refractive error, and other myopia endophenotypes was used to identify articles for this review (available at http://www.ebi.ac.uk/gwas/home). Articles included are summarized in Table 1.

High myopia GWAS

The first published GWAS in myopia examined a Japanese population with 297 cases of pathological myopia (defined as axial length > 26 mm) and 977 controls from the general population [31]. The strongest association was located at 11q24.1, approximately 44 kb upstream of the BLID gene, and conferred odds of higher myopia of 1.37 (95% confidence interval (CI) 1.21–1.54). Subsequently, a meta-analysis of two ethnic Chinese cohorts, published in 2010, was performed for 287 cases of high myopia (defined as ≤ −6 D) and 911 controls [32]. The strongest association was an intronic SNP within the CTNND2 gene on 5p15.2. However, neither of these initial associations met the conventional GWAS threshold ($p \leq 5 \times 10^{-8}$) for statistical significance.

Li et al. also studied an ethnic Chinese population inclusive of 102 high-grade myopia cases (defined as ≤ −8 D with retinal degeneration) and 335 controls [33]. The strongest association ($p = 7.70 \times 10^{-13}$) was a high-frequency variant located in a gene desert within the MYP11 myopia linkage locus on 4q25 [34]. In a similar ethnic Han Chinese population of 419 high myopia cases (≤ −6 D) and 669 controls, Shi et al. identified the strongest association ($p = 1.91 \times 10^{-16}$) at an intronic, high-frequency variant within the MIPEP gene on 13q12 [35]. Although these studies attempted replication in independent cohorts, their results, published in 2011, have not been replicated in GWAS comprising of individuals of similar ethnic background, phenotypic definition, or study design.

Table 1. Summary of published GWAS in myopia.

Study	Year	Association count	Region of associations	Genes implicated
Nakanishi H et al. [31]	2009	1[a]	11q24.1	BLID
Li YJ et al. [32]	2011	1[a]	5p15.2	CTNND2
Li Z et al. [33]	2011	2	4q25	MYP11 linkage locus
Shi Y et al. [35]	2011	1	13q12	MIPEP
Meng W et al. [38]	2012	64[a]	8p23	MYP10 linkage locus
			10q21.1	MYP15 linkage locus
Shi Y et al. [36]	2013	5	13q12.12	VIPR2
			8q24.12	SNTB1
Khor CC et al. [37]	2013	2	2q22.3	ZFHX1B
			8q24.12	SNTB1
Hysi PG et al. [39]	2010	1	15q25.1	RASGFR1
Solouki AM et al. [40]	2010	1	15q14	GJD2
Stambolian D et al. [41]	2013	1	16p13.3	RBFOX1
Verhoeven VJ et al. [42]	2013	26	BICC1, BMP2, BMP3, CACNA1D, CD55, CHD7, CHRNG, CNDP2, CYP26A1, GJD2, CRIA4, KCNJ2, KCNQ5, LAMA2, MYO1D, PCCA, PRSS56, RASGRF1, RDH5, RORB, SIX6, TOX, ZIC2, ZMAT4	
Kiefer AK et al. [43]	2013	22	BMP3, BMP4, DLG2, DLX1, GJD2, KCNMA1, KCNQ5, LAMA2, LRRC4 C, PABPCP2, PDE11A, PRSS56, RASGFR1, RBFOX1, RDH5, RGR, SFRP1, SHISA6, TJP2, TOX, ZBTB38, ZIC2	

[a]Associations not reaching conventional GWAS threshold ($p \leq 5 \times 10^{-8}$) for statistical significance.

In 2013, two papers reported replicated loci for high myopia in Asian populations. Shi et al. studied a Han Chinese population of 665 cases with high myopia (≤ −6 D) and 960 controls [36]. Following two-stage replication in three independent cohorts, the most significantly associated variant ($p = 8.95 \times 10^{-14}$) was in the VIPR2 gene within the MYP4 locus, and three further variants all reaching genome-wide significance were identified within the same linkage disequilibrium block in the SNTB1 gene ($p = 1.13 \times 10^{-8}$ to 2.13×10^{-11}). Khor et al. reported a meta-analysis of four GWAS of East Asian ethnicity totaling 1603 cases of 'severe' myopia (based on either refractive error or axial length) and 3427 controls [37]. After replication analysis, the SNTB1 gene was confirmed and a novel variant within the ZFHX1B gene (also known as ZEB2) reached genome-wide significance ($p = 5.79 \times 10^{-10}$).

In European populations, probably illustrating the lower prevalence of high myopia, there has only been one case–control GWAS from a French population, published in 2012. In this study of 192 high myopia cases (≤ −6 D) and 1064 controls, a suggestive association was identified within the MYP10 linkage locus, 3 kb downstream of PPP1R3B; however, this did not reach genome-wide statistical significance and the study failed to replicate any of the previously reported loci [38].

Refractive error quantitative GWAS

Greater success has been achieved by considering refractive error as a quantitative treat, including all subjects in population-based studies rather than a selected clinic-based sample of highly affected individuals. In 2010, the first two GWAS for refractive error were published, both in European populations; a British discovery cohort of 4270 individuals [39] and a Dutch discovery cohort of 5328 individuals [40], with replication in over 10,000 individuals from the two discovery cohorts and a smaller shared pool of replication samples. Two loci surpassing the GWAS threshold were identified near the RASGFR1 gene on 15q25.1 ($p = 2.70 \times 10^{-09}$) and the other near GJD2 on 15q14 ($p = 2.21 \times 10^{-14}$). Subsequently, in 2013, a relatively small meta-analysis was performed on 7280 individuals from five cohorts with refractive error, inclusive of various ethnic populations across different continents. Replication was then undertaken in 26,953 samples [41]. A novel variant reaching the GWAS threshold identified within the RBFOX1 gene on chromosome 16 was identified ($p = 3.9 \times 10^{-9}$).

The field made a major breakthrough in 2013 when two major GWAS meta-analysis studies were published. The Consortium for Refractive Error and Myopia (CREAM) is an international collaborative initiative between researchers studying cohorts of both European and Asian descent. A classic meta-analysis of the GWAS results for a linear regression between genotype and spherical equivalent of refractive error was performed for 35 participating centers, comprising 37,382 individuals of European descent and 12,332 of Southeast Asian ancestry [42]. High statistical power was achieved by this large sample size, enabling replication of the two loci previously identified and identification of 22 novel loci at genome-wide significance (Figure 3): BICC1, BMP2, BMP3, CACNA1D, CD55, CHD7, CHRNG, CNDP2, CYP26A1, GJD2, CRIA4, KCNJ2, KCNQ5, LAMA2, MYO1D, PCCA, PRSS56, RASGRF1, RDH5, RORB, SIX6, TOX, ZIC2, and ZMAT4.

A contemporaneous GWAS by the direct-to-consumer genomics company 23andMe (Mountain View, CA, USA) using a survival analysis was performed on 55,177 individuals of European descent using the phenotype of reported myopia and reported 'age of spectacle wear' as a proxy for myopia severity [43]. The authors identified 20 novel loci: BMP3, BMP4, DLG2, DLX1, GJD2, KCNMA1, KCNQ5, LAMA2, LRRC4C, PABPCP2, PDE11A, PRSS56, RASGFR1, RBFOX1, RDH5, RGR, SFRP1, SHISA6, TJP2, TOX, ZBTB38, and ZIC2. Contrary to many researchers' expectations, the authors identified highly comparable genetic associations to those obtained using the carefully and expensively collected refractive error data in population-based samples in the CREAM consortium. Of the 22 loci discovered by CREAM, 14 were replicated by 23andMe, whilst 16 of the 20 loci identified by 23andMe were confirmed by CREAM. Surprisingly, the same 25 genetic loci were identified in both studies with consistent direction of effect despite analysis on different scales, namely diopters for CREAM (more negative on the scale indicative of more myopia) and hazard ratios (higher positive hazard ratios indicative of more severe myopia) for 23andMe [44,45].

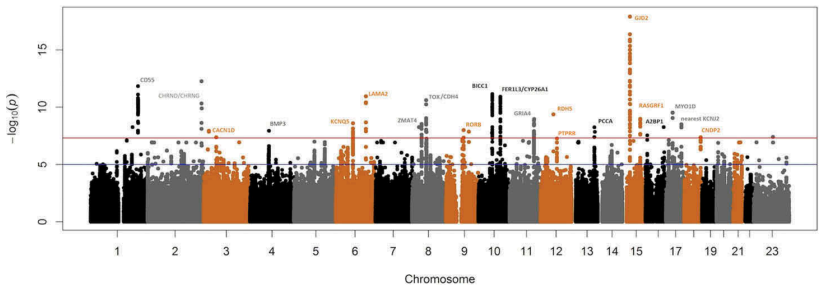

Figure 3. Manhattan plot of genetic associations for refractive error in the CREAM combined GWAS meta-analysis. -$\log_{10}$-transformed p values for all SNPs. The upper horizontal line indicates the $p < 5.0 \times 10^{-8}$ threshold, the lower horizontal line indicates a p value $< 1 \times 10^{-5}$ (data from [42]).

GWAS and myopia endophenotypes

The most common type of myopia is axial myopia (lens-induced or lenticular myopia is seen in old age due to early nuclear cataract), and as such the axial length of the eye is a major determinant of refractive error. A number of researchers have therefore used this myopia proxy or 'endophenotype' in genetic association studies. The first of these, published in 2012, examined 4944 individuals of East and South East Asian ancestry [46]. One locus on 1q41 containing the zinc-finger pseudogene ZC3H11B reached genome-wide significance ($p = 4.38 \times 10^{-10}$), although replication was not performed.

A much larger GWAS meta-analysis for axial length comprising 12,531 Europeans and 8216 Asians was published in 2013 [47]. Eight, novel genome-wide significant loci were identified (RSPO1, C3orf26, LAMA2, GJD2, ZNRF3, CD55, MIP, ALPPL2) and the study also replicated the ZC3H11B gene. Relevantly, five of these loci had been previously associated in refractive error GWAS.

Shared determination of an individual's axial length and corneal curvature was identified in the Avon Longitudinal Study of Parents and Children and Singapore Chinese Eye Study, suggesting that shared genetic variants control these two parameters that contribute to the eye's focus [48]. A number of relatively small GWAS have been performed for corneal curvature in individuals of varying ancestry with identified associations including FRAP1, PDGFRA (also associated with eye size), CMPK1, and RBP3 [49–52]. More recently, Miyake et al. published a two-stage GWAS for three myopia-related traits: axial length, corneal curvature, and refractive error [53]. The study was performed on 9804 Japanese individuals with trans-ethnic replication in Chinese and Caucasian individuals. A novel gene, WNT7B, was identified for axial length ($p = 3.9 \times 10^{-13}$) and corneal curvature ($p = 2.9 \times 10^{-40}$), whilst the previously reported association with GJD2 and refractive error was replicated.

Pathways implicated from GWAS in myopia

Identifying genes associated with myopia is just the first step in maximizing information from GWAS to improve understanding of myopia etiology. Some individual biological mechanisms can be implicated from genes associated, but pathway analysis enables a more comprehensive, systems biology approach to understanding how associated genetic variants can ultimately influence ocular growth. Pathway analysis, however, does rely on previously published work on the functionality of certain genes.

Functional pathways (or ontological classifications) implicated by the large GWAS on myopia to date have been clear and reproducible [54]. Interestingly, they provide credible evidence that the genetic architecture is fairly consistent between two continental populations (European and Asian). As with many GWAS, the variants identified have not necessarily fallen within a gene but likely functional implications to proximal, relevant genes have been inferred. Although this is reasonable, there are other known factors, such as long-range distance equilibrium, which may mean alternate genes or pathways could equally be involved. Biological processes indicated from the CREAM meta-GWAS include neurotransmission (GRIA4), ion transport (KCNQ5), retinoic acid metabolism (RDH5), extracellular matrix remodeling (LAMA2, BMP2), and eye development (SIX6, PRSS56) [42]. The 23andMe meta-GWAS similarly implied extracellular matrix remodeling (LAMA2, ANTXR2), the visual cycle (RDH5, RGR, KCNQ5), neuronal development (KCNMA1, RBFOX1, LRRC4C, NGL-1, DLG2, TJP2), eye and body growth (PRSS56, BMP4, ZBTB38, DLX1), and retinal ganglion cell projections (ZIC2, SFRP1) [43]. Hysi et al. reported that plasma membrane, cell–cell adhesion, synaptic transmission, calcium ion binding, and cation channel activity were significantly overrepresented in association with refractive error in two British cohorts [54].

Whilst the biological processes implied by these genes may at first seem disparate, the protein products and end functions can be highly correlated. By examining known protein–protein interactions, researchers have identified that in fact many of the genes implicated from the meta-GWAS in myopia are related to cell cycle and growth pathways such as the MAPK and transforming growth factors beta (TGF-β)/SMAD pathways, as shown in Figure 4 [45]. This network analysis can

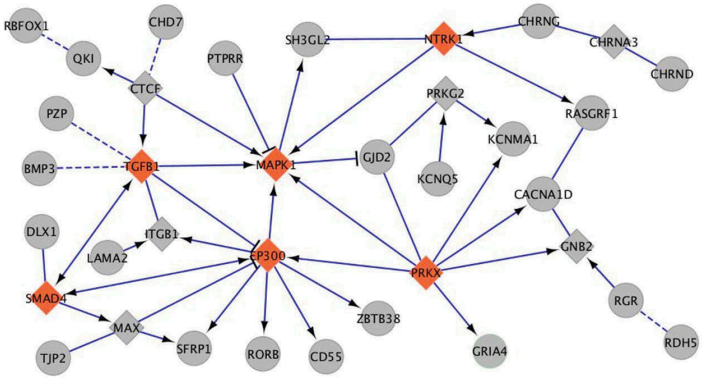

Figure 4. Network connections of genes associated with myopia. Genes identified in GWAS are in round grey nodes, linker elements in square nodes, MAPK & TGF-β/SMAD pathway elements are in orange, solid blue edges identify protein-protein interactions and dashed blue edges symbolize corregulation relationships. (data from [45]).

provide greater insight into how refractive error develops, although it must be acknowledged that the risk loci identified from GWAS have not been shown to be causative in functional studies and therefore any pathway analysis is speculative.

GWAS and gene–environment interactions

Although myopia is a highly heritable trait, it is known that environmental factors are highly influential in determining myopia risk and must be driving the recent epidemic rise in prevalence [1]. One of the most influential and highly replicated factors is education [4,55–58]; research suggests that those going onto higher education have double the myopia prevalence than those who leave school after primary education [4]. Education has therefore been the primary environmental choice for gene–environment (G×E) interaction analyses in myopia. G×E studies acknowledge that individuals of a differing genotype may respond to environmental variation in differing ways; for example, in some individuals an environmental exposure may trigger a certain gene to be unregulated whilst in others there is no effect. This method of analysis therefore has the potential to show how prior identified variants are modified by environmental exposure, but may also identify variants that were previously only suggestively associated with the disease of interest.

Two research groups have examined this phenomenon by using the myopia-associated variants from the CREAM meta-GWAS. In the first, individuals of European descent were categorized as having completed a primary, intermediate, or higher education, and then assigned a polygenic risk score based on the 26 myopia-associated variants from the CREAM meta-GWAS [59]. There appeared to be an interaction between the effect of higher education and having a high genetic risk score; the odds ratio for myopia in those with high genetic risk completing higher education was 51.3 (95% CI 18.5–142.6) compared to an odds ratio of 7.2 (95% CI 3.1–17.0) if only primary education was achieved. The combined effect of the two risk factors was far greater than the sum of the separate factors (synergy index = 4.2, 95% CI 1.9–9.5), providing evidence that an interaction effect between an environmental factor and an individual's genotype was occurring. A similar analysis was performed on five Singaporean cohorts; this analysis identified three genes (DNAH9, GJD2, and ZMAT4-SFRP1) that were strongly associated with myopia in individuals achieving higher secondary or university education but that were either borderline or not statistically significant in individuals achieving lower secondary education or below [60].

Implications from GWAS in myopia

GWAS have enabled considerable progress in our understanding of what genetic variants are associated with myopia; the number of variants identified in the recent meta-GWAS far exceeds those identified by linkage and candidate gene studies. However, the high heritability of refractive error and myopia, which is between 70% and 80% [5–15], is only partly explained by the variants so far identified. In a European cohort, the variants identified by the CREAM meta-GWAS explain only 3.4% of the variance of refractive error [42]. This means approximately 75% of the expected heritability is 'missing', a recurrent problem in GWAS of complex diseases [27].

In an attempt to identify missing variants for complex diseases, sample sizes need to be maximized. It is well known that small sample sizes reduce power and accuracy in capturing genetic associations. Since the publication of the major meta-GWAS in refractive error, two studies, of relatively small size (<1900 individuals), have failed to fully replicate results [61,62]. Conversely, results from high-grade GWAS in refractive error were not replicated by the meta-analysis of CREAM; this may be due to phenotypic or genetic heterogeneity, or, more likely, lack of statistical power [63,64]. It must be acknowledged that underpowered GWAS may produce spurious or false-positive results.

GWAS have confirmed that myopia is highly polygenic with significant variation in the allelic spectrum of identified loci; that is to say, the minor allele frequency (MAF), indicative of how common the polymorphism is within a population, varied extensively within both the CREAM and 23andMe GWAS [45]. However, the majority of variants had only a small effect on phenotypic variants with the highest effect sizes limited to the variants with the lowest MAF (Figure 5). GWAS, in its current

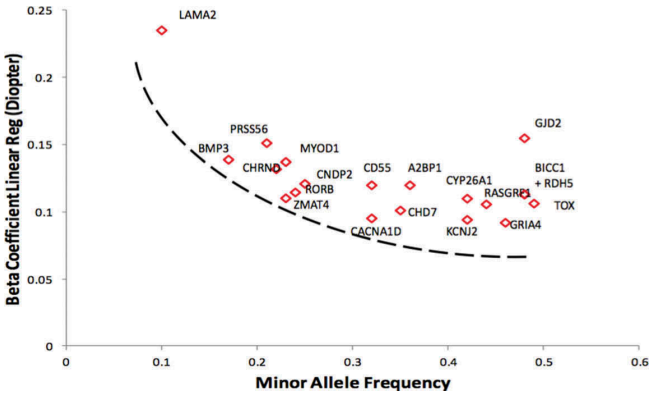

Figure 5. Minor allele frequency against effect size for the significant variants identified in the CREAM GWAS (data from [45]).

form, is limited to assessing associations between a phenotype and common genetic variants. This means variants of lower allelic frequency (rare variants) but potentially large effect sizes have not been investigated.

We can therefore infer that GWAS will never fully explain all the expected heritability from twin studies. A better means of estimating how much variance can potentially be explained by common genetic variation is to perform a genome-wide complex trait analysis or SNP-based heritability [65–67]. This technique allows estimation of how much intersubject variation of a trait can be explained by all the available SNPs. The number of SNPs that have been genotyped or imputed for that individual limits the method, and therefore, the SNP-based heritability corresponds to a lower-bound estimate. In a pediatric, British cohort, SNP-based heritability was found to remain stable over childhood and, after adjustment for the lack of cycloplegia on the study participants, the SNP heritability, averaged over childhood, was 0.35 (standard error = 0.09) [68]. This would suggest that common genetic variants could explain 35% of variance, approximately half of the estimated heritability from twin studies. For comparison, the authors point out that the variance explained by nongenetic risk factors, such as time indoors and time spent reading, is less than 1%. It therefore remains possible that more common variants of small effect could be found using common SNP-based association techniques and that there is merit in continuing to use the technique with ever larger sample sizes in an attempt to capture more genetic variants. Rarer variants (in the order of MAF = 1–5%), with potentially greater effect on phenotypic variation, may be identified with improved accuracy using the greater coverage conferred with the 1000 Genomes haplotype map and larger sample sizes.

One of the key questions for clinicians is whether our current understanding of myopia genetics allows prediction of future myopia status for children. Predicting disease risk is most commonly performed using receiver-operating characteristic curves [69]. This is a plot of the sensitivity of a test against 1-specificity of a test using all possible thresholds of high risk versus low risk. The area under the curve (AUC) is equal to the probability that a randomly identified individual with the disease has a higher risk than a randomly selected healthy individual. An AUC, or C-statistic, is given as a fraction with a perfect test yielding an AUC of 1 and a test with no discriminatory power having an AUC of <0.5. The predictive accuracy of genetic-risk models varies extensively between diseases, but to date confers little benefit over nongenetic risk prediction models [70]. AMD has been an exception, with an AUC of 0.82 for the full combination of associated genetic variants identified through GWAS [71]. The utility of prediction models for AMD in clinical practice has been further tested by adding in phenotypic and demographic information, such as age and smoking, which increases the AUC to 0.87 [72]. However, in the majority of disease phenotypes an AUC of 0.5–0.7 is more commonly achieved [70], which confers little predictive value, and this is true for myopia at our current level of understanding of the genetic architecture.

To increase the potential for predicting genetic risk entails greater understanding of the genetic architecture of myopia. As discussed, we estimate there are more common genetic variants to be identified and given that very low-frequency variants are unlikely to contribute greatly to population variance, we can be optimistic that most of the phenotypic variations in myopia could be explained by common genetic variants [66]. However, there are other genetic factors contributing to heritability. Genetic risk is a complex result of common genetic variation, rare genetic variation, G×E interactions, gene–gene interactions, epigenetics, and a host of other variations in our genetic make-up. Rare genetic variation requires new analysis techniques and more detailed sequencing of the genome of study participants. Fortunately, next-generation sequencing has provided reduced cost of high-throughput, high-coverage genotyping, enabling whole-exome and whole-genome examination. Higher-density SNP chips have also been developed, either for higher coverage of the genome or exome-specific. This means greater coverage of the genome but also increased accuracy as the reliance on imputation, typically poor for rare SNPs, is reduced. As methods for analyzing these vast data sets are refined, this will dramatically increase the potential for identification of rare variants and has already proved successful [73,74]. Interactions between our environment and our genome have already proved informative in myopia, whilst interactions between genes and other genetic architectural analysis techniques hold promise for the future.

Expert commentary

GWAS in myopia have undoubtedly transformed our understanding of the genetic architecture of this complex trait. This is very relevant as myopia, already the most common eye condition, is increasing in prevalence throughout world. In light of the fact that myopia is a highly heritable trait, deeper understanding of how genetic variation leads to development of myopia is increasingly necessary.

The genetic variants identified from the major GWAS in myopia have been clear and reproducible, providing credible evidence for their association. Biological processes indicated by the identified associations include neurotransmission, ion transport, retinoic acid metabolism, extracellular matrix remodeling, eye development, the visual cycle, neuronal development, eye and body growth, and retinal ganglion cell projections. Enrichment analysis suggests plasma membrane, cell–cell adhesion, synaptic transmission, calcium ion binding, and cation channel activity appear to be significantly overrepresented in refractive error. Many of the genetic associations are related to cell cycle and growth pathways such as the MAPK and TGF-β/SMAD pathways.

However, only around 3% of myopia variance is explained by the genetic variants identified to date. SNP-based heritability analysis suggests common genetic variation accounts for approximately 35% of myopia variance. Therefore, there is more work to be done in an effort to capture all associated common genetic variants. This requires larger samples and improved genotyping to reduce the burden on imputation, which ultimately can lead to poor ability to capture associated variants or conversely false-positive results. Alternate analysis techniques and

proxy endophenotypes are being explored in an effort to further increase our ability to identify these variants. The interplay between genes, and genes and environment is being examined in relation to myopia with some success, shedding new light on how genetic variation may be modified and ultimately lead to myopia development in different individuals. It is also important to acknowledge that twin-based estimates of heritability are much higher, at 70–80%, and suggest that genetic factors other than common genetic variation may play a role.

This paper provides a review of our current understanding into the genetics of myopia. There is much work still to be done, and this will be required before our ability to predict future development of myopia becomes a reality. GWAS provides the first step in our ability to identify novel loci and functional pathways. This must then be built upon with other genetic association modalities and the use of both animal models, although notably to date there are few genetic animal models for myopia, and pharmacological studies. Only then can researchers begin to target myopia development and reduce the burden from this common, sight-threatening disease.

Five-year view

Despite significant progress in recent years, we still can only explain a very small proportion of myopia variance by genetic factors. In the next five years, new approaches to try and capture more of the genetic variance will be employed. Firstly, the simple approach of 'bigger is better' should be employed; ever-larger meta-analysis of GWAS from across the globe must be utilized in a collaborative format to increase the research community's ability to find genes. This may involve using phenotype data that extends beyond the traditional modality of spherical equivalent into combining GWAS performed on proxy phenotypes and endophenotypes.

Secondly, a more detailed interrogation of the genome is required to identity rare genetic variants, and notably these variants may play a more significant role in myopia risk. This can be brought about through a number of existing methods. Using currently genotyped data, the improved imputation capacity conferred by haplotype maps such as 1000 Genomes should be employed to reduce imputational errors leading to false-negative and false-positive associations; notably both of the major GWAS on myopia to date are based on HapMap imputed data. An alternate method is employment of the improved genotyping ability that can be achieved with high-density chips and next-generation sequencing. These modalities achieve greater coverage of the genome, reduced genotyping errors, and a reduced reliance on imputation. Although there are many obstacles to overcome such as data storage requirements for these vast files, refinement of analysis techniques, and establishment of how results are interpreted, they do provide a means to attempt to capture the known missing heritability in myopia.

Finally, alternate means of understanding the genetic architecture of myopia should be employed – extending beyond simple association methods to explore interactions and the effect of other 'omics'. This may include incorporation of transcriptomics or metabolomics, for example, with existing association methods to allow a more systems biology-based approach to understanding how genetic variation ultimately leads to myopia development.

Key issues

- Myopia is the most common eye condition worldwide, and the prevalence is increasing.
- Myopia has a complex trait with strong environmental risk factors such as education and lack of time spent outdoors, and a high heritability of 70–80%.
- Genome-wide association studies (GWAS) have enabled rapid association of common genetic variants with disease since 2005 in various traits, most successfully in AMD.
- Case–control high myopia GWAS have been largely performed in Asian populations with a number of genetic variants identified.
- The largest identification of variants for myopia was performed in two GWAS, by the Consortium for Refractive Error and Myopia (CREAM) consortium and 23andMe, published in 2013; the 26 genetic loci by CREAM identified explain less than 5% of myopia variance.
- Functional pathways implicated by the genetic variants identified for myopia include plasma membrane, cell–cell adhesion, synaptic transmission, calcium ion binding, and cation channel activity, with many of the genetic associations related to cell cycle and growth pathways.
- Gene by environment analyses suggest interaction effects do occur between the currently identified genetic variants and higher education, one of the strongest risk factors for myopia.
- In an attempt to capture more of the genetic variants for myopia, with the ultimate of aim of enabling risk prediction and developing targeted interventions, larger sample sizes are required with deeper coverage of the genome.

Declaration of interest

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•• of considerable interest

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Chapter 2 | Aims of Thesis

Aim: To examine the environmental and genetic factors for myopia primarily using the Twins Early Development Study (TEDS), with additional analyses in the TwinsUK and EUREYE studies, and collaborative analyses with the European Eye Epidemiology (E^3) consortium and Consortium for Refractive Error and Myopia (CREAM).

Hypotheses:

What is the current burden of refractive error and myopia in Europe and is there evidence for a rising prevalence?

Do factors in modern day childhood in the UK, such as less outdoor play, increased time indoors on computer games, or changes in education style, explain the potentially increasing levels of myopia?

Can a detailed examination of cognition, intelligence and education explain what aspects are most predisposing to myopia development?

Are the established associations of myopia with higher educational attainment and intelligence due to shared genetic effects?

Is there evidence of a gene-environment interaction with risk factors for myopia?

Objectives:

1. *Obtain prevalence estimates for myopia in Europe and explore if there is evidence for a rising trend, with consideration of potential causes*
2. *Explore typical age of onset of myopia in UK cohorts and what this can tell us about future severity of myopia*
3. *Obtain an estimate of the current burden and age of onset of childhood-onset myopia in the UK*
4. *Examine the relationship between refractive error and educational, behavioural and cognitive traits*
5. *Explore the aetiology behind the protective effect of time outdoors using existing datasets with information on sunlight and vitamin D*
6. *Determine refractive error heritability at age 18 and investigate the relative effect of genes and environment using a classic twin study*
7. *Extended twin modelling and techniques using genotype data will be used to assess potential shared genetic effects underlying myopia and previously associated traits if replicated (eg. intelligence)*

8. *Perform a genome wide association study (GWAS) for refractive error; examine the role of common variants identified from adult GWAS in childhood-onset myopia*
9. *Assess gene-environment interactions in myopia*

The findings of this thesis will be presented as a thesis incorporating publications. In *Chapter 3* I present my findings on the changing epidemiology (and age of onset) of myopia in Europe and the UK through the presentation of three published papers. In *Chapter 4* I explore environmental factors for myopia through one paper currently in review and one published paper; the first on early life factors for myopia and the second on the association between myopia and light (UVB), vitamin D levels and polymorphisms in vitamin D pathway genes. In *Chapter 5* I will present my work into the genetic aetiology of myopia with the results of a classic twin study and genome-wide association study in TEDS and two publications (one in review and one published), including an examination of the genotypic correlation between myopia and intelligence and a meta–analysis exploring the interaction between 'myopia genes' and two environmental associations (near work and time outdoors). In *Chapter 6* I will draw together the findings reported in each chapter and discuss overall implications of this thesis.

Chapter 3 | The changing epidemiology of myopia in Europe and the United Kingdom

3.1 Introduction

In this chapter I present three published papers illustrating the epidemiology of myopia in the UK and Western Europe. The first paper is a meta-analysis I performed of refractive error prevalence across Europe in over 60,000 individuals, as part of the European Eye Epidemiology Consortium (E^3). The second, a similar meta-analysis of the E^3 consortium that I performed, but with a focus on myopia, evidence for a rising myopia prevalence, and an exploration of the relationship with education. In the final paper I examine the age of myopia onset in a UK adult twin cohort (TwinsUK); where I also examine the association between age of onset and myopia severity, and evidence for a rising prevalence of myopia.

3.2 Prevalence of refractive error in Europe

This chapter is presented as a published paper and is an exact copy of the following journal publication:

Katie M Williams, Virginie JM Verhoeven, Phillippa Cumberland, Geir Bertelsen, Christian Wolfram, Gabriëlle HS Buitendijk, Albert Hofman, Cornelia M van Duijn, Johannes R Vingerling, Robert WAM Kuijpers, René Höhn, Alireza Mirshahi, Anthony P Khawaja, Robert N Luben, Maja Gran Erke, Therese von Hanno, Omar Mahroo, Ruth Hogg, Christian Gieger, Audrey Cougnard-Grégoire, Eleftherios Anastasopoulos, Alain Bron, Jean-François Dartigues, Jean-François Korobelnik, Catherine Creuzot-Garcher, Fotis Topouzis, Cécile Delcourt, Jugnoo Rahi, Thomas Meitinger, Astrid Fletcher, Paul J Foster, Norbert Pfeiffer, Caroline CW Klaver, Christopher J Hammond (on behalf of the European Eye Epidemiology Consortium (E^3)). Prevalence of refractive error in Europe: the European Eye Epidemiology (E^3) Consortium. *Eur J Epidemiol*. 2015 Apr;30(4):305-15. doi: 10.1007/s10654-015-0010-0. Epub 2015 Mar 18

Eur J Epidemiol
DOI 10.1007/s10654-015-0010-0

OPHTHALMIC EPIDEMIOLOGY

Prevalence of refractive error in Europe: the European Eye Epidemiology (E^3) Consortium

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Abstract To estimate the prevalence of refractive error in adults across Europe. Refractive data (mean spherical equivalent) collected between 1990 and 2013 from fifteen population-based cohort and cross-sectional studies of the European Eye Epidemiology (E^3) Consortium were combined in a random effects meta-analysis stratified by 5-year age intervals and gender. Participants were excluded if they were identified as having had cataract surgery, retinal detachment, refractive surgery or other factors that might influence refraction. Estimates of refractive error prevalence were obtained including the following classifications: myopia ≤ -0.75 diopters (D), high myopia ≤ -6D, hyperopia ≥ 1D and astigmatism ≥ 1D. Meta-analysis of refractive error was performed for 61,946 individuals from fifteen studies with median age ranging from 44 to 81 and minimal ethnic variation (98 % European ancestry). The age-standardised prevalences (using the 2010 European Standard Population, limited to those ≥ 25 and <90 years old) were: myopia 30.6 % [95 % confidence interval (CI) 30.4–30.9], high myopia 2.7 % (95 % CI 2.69–2.73), hyperopia 25.2 % (95 % CI 25.0–25.4) and astigmatism

On behalf of the European Eye Epidemiology Consortium (E^3).

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23.9 % (95 % CI 23.7–24.1). Age-specific estimates revealed a high prevalence of myopia in younger participants [47.2 % (CI 41.8–52.5) in 25–29 years-olds]. Refractive error affects just over a half of European adults. The greatest burden of refractive error is due to myopia, with high prevalence rates in young adults. Using the 2010 European population estimates, we estimate there are 227.2 million people with myopia across Europe.

Keywords Refractive error · Myopia · Epidemiology · Prevalence · Consortium

Introduction

Refractive error occurs when there is failure of the eye to correctly focus rays of light from an object onto the retinal plane. The resultant image perceived by the individual is blurred and refractive correction is required in order to see clearly. Refractive error can be divided into myopia ('short or near-sightedness'), hyperopia ('long or far-sightedness') and astigmatism. In myopia, light is focussed to a point anterior to the retina as a result of excessive refraction at the cornea or lens, or, more commonly, an increased length of the eye ('axial myopia'). In hyperopia, the reverse occurs with an image forming posterior to the retinal plane as a result of either inadequate refraction or a short axial length. In astigmatism, the refractive power of the eye is uneven across different meridians.

Refractive error requires detection and treatment in the form of glasses, contact lenses or, more recently, refractive surgery. These clinical services are readily available in most European countries, although they come with significant financial implications to both national health care systems and to individuals [1]. However, uncorrected refractive errors are still responsible for up to 42 % of the cases of visual impairment worldwide [2], and remain prevalent even in high income countries [3–6]. Uncorrected refractive error in both low and high-income countries has significant economic implications in terms of potential lost productivity [7].

The magnitude of refractive error in developed countries within individuals of European descent has been estimated by the Eye Diseases Prevalence Research Group, 10 years ago, and the US National Health and Nutrition Examination Survey (NHANES) data [3, 8]. However, the estimate of refractive error burden in Europe was based on a single cohort [9]. The European Eye Epidemiology (E^3) consortium is a collaborative initiative between thirty-three cohort studies across Europe, to share and meta-analyse epidemiological data on eye disease in adults. The aim of the current study was to provide more current and precise estimates of the prevalence of refractive error across Europe.

Materials and methods

Studies and participants

To date, E^3 has data from thirty-three studies with a range of ophthalmic data on approximately 124,000 individuals from population-based and case–control studies. This study drew on the fifteen E^3 population-based cohort and cross-sectional studies that collected refractive error data ($n = 68{,}350$). As described in Table 1, participants included in this meta-analysis were largely from Northern and Western Europe, mainly of middle to late age, and refractive error measurements were performed between 1990 and 2013. Three studies recruited participants nationally and the remaining twelve recruited from a local population. Further detail on individual study design and sampling method is provided in the supplementary information; broadly, the majority of study samples were obtained by identification of potential participants (within defined age bands and/or regions) using local registries, with some studies using random sampling ($n = 3$). All studies adhered to the tenets of the Declaration of Helsinki, and relevant local ethical committee approvals with specific study consent were obtained.

Inclusion and exclusion criteria

Studies in the E^3 consortium were eligible for inclusion in this analysis if they were population-based, and data on refraction, together with age at measurement and year of birth, were available. Study participants were excluded if they were identified as having had cataract surgery, retinal detachment, refractive surgery or other factors that might influence refraction (e.g. keratoconus), at the discretion of each study's analysis team.

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Demographic and outcome variables

All included studies measured non-cycloplegic refraction (i.e. no dilating drops were used) using the technique of subjective refraction, autorefraction or a combination of focimetry (measuring an individuals glasses) or autorefraction followed by subjective refraction (Table 1). Participant's spherical equivalent (SE) was considered as the mean SE of the two eyes calculated using the standard formula (SE = sphere + (cylinder/2)). Refractive error was categorized using the following definitions: myopia ≤ -0.75 diopters (D), low myopia ≤ -0.75 to > -3D, moderate myopia ≤ -3D to > -6D, high myopia ≤ -6D, hyperopia ≥ 1D, high hyperopia ≥ 3D and astigmatism ≥ 1D. Definitions of myopia vary in the literature; the cut-off of -0.75D was chosen as unaided visual acuity at this level approximates 0.3 LogMAR (Logarithm of the Minimum Angle of Resolution) [10], a commonly used driving standard, and this has been used in recent international meta-analyses of the genetic epidemiology of refractive error and myopia [11].

Differences in age (in 5 year age bands from ≥ 15 to ≥ 90 years), gender (male/female) and geographical European region were examined. Geographical variations in the prevalence of myopia were investigated by dividing countries in three areas (Northern, Western and Southern Europe) according to the United Nations Geoscheme [12]. Information on ethnicity, when available, was recorded using a modified classification system based on genetic ancestry [13].

Statistical analysis

Study specific summary data were obtained. A random effects meta-analysis was performed for spherical equivalent and repeated for refractive classifications overall and stratified by age. This enabled calculation of pooled estimates of refractive error prevalence, with studies weighted by sample size and between-study variance and a summary estimate standard error calculated from the inverse sum of the adjusted weights. A random effects model was chosen over a fixed effects model, to allow for heterogeneity in study design characteristics.

Age-standardised prevalences were calculated using the following steps: firstly, age-specific prevalences were estimated using random-effect meta-analyses. Secondly, an age-standardisation with adjustments to age-specific estimates according to the European Standard Population 2010 was performed [14]. This enabled refractive error prevalence estimates that are representative for the European population, with appropriate weighting to the age demographic distribution of Europe.

Subsequent random effects meta-analyses were performed with stratification by age and gender, and subsequently age and geographical region, with differences between groups evaluated using ANOVA tests.

Statistical analysis was performed using Stata version 13.1 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). Graphical outputs were obtained using either Stata or ggplot2 [15] in R (R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org).

Results

Fifteen studies contributed a total of 61,946 individuals after exclusions (Fig. 1). The median age of the included populations ranged from 44 to 78 years old (Table 1). There was a slight female predominance in the combined study (57.6 % females). Data on ethnicity was only available for 50 % of participants, and in these there was minimal ethnic diversity (98 % European ancestry), so no further analysis of ethnicity was carried out.

The distribution of refractive error displayed a leptokurtotic distribution (Fig. 2), with a median spherical equivalent of 0.56D (range -25.13–22.19). The distribution was asymmetric with a greater frequency of individuals with a negative refractive error.

Given there were only 314 participants aged 15–24 years and 156 >90 years of age, subsequent analyses are limited to those aged ≥ 25 and <90 years ($n = 61{,}476$). The overall myopia prevalence in our meta-analysis was 24.2 % (95 % confidence interval (CI) CI 19.9–28.5), with a European age-standardised myopia prevalence of 30.6 % (95 % CI 30.4–30.9; Table 2). Myopia was most common in younger participants [peaking at 47.2 % (95 % CI 41.8–52.5) in those aged 25–29 years], almost double the prevalence of those of middle and older age [27.5 % (95 % CI 23.5–31.5) in those aged 55–59 years; Fig. 3a]. Point estimates of myopia prevalence in those aged 15–19 years were 27.4 % (95 % CI 17.0–37.8), increasing to 34.2 % (95 % CI 27.9–40.6) in those aged 20–24 years. All degrees of myopia followed a similar pattern of higher prevalence in the younger cohorts, lower prevalence in the middle aged and more elderly participants, and an increase in the very eldest participants, albeit with wide CIs, most likely related to cataract development. Age-standardised prevalence of high myopia across all age groups was 2.71 % (95 % CI 2.69–2.73), with 3–5 % of young to middle-aged individuals affected and 1–2 % of older individuals (Fig. 3b).

Overall prevalence of hyperopia was 34.7 % (95 % CI 27.9–41.6), with an age-standardised prevalence of 25.2 % (95 % CI 25.0–25.4). There was less hyperopia in young participants [6.4 % (95 % CI 3.8–9.0) in those aged

Table 1 Description of the 15 European Eye Epidemiology consortium studies included in this meta-analysis of refractive error

Study	Data collection period	Study design	Total with refraction	Refraction method	Exclusions (cataract surgery)	Total included	Median age, years (range)	Gender, % female	Ethnicity, % European (% Unknown)	Crude myopia prevalence, %	Crude hyperopia prevalence, %
Northern Europe											
1958 British birth cohort, UK	2002–2003	Population-based birth cohort (N)	2502	Autorefraction	7 (0)	2495	44 (44–46)	51.7	98.0 (9.2)	48.7	8.8
EPIC-Norfolk, UK	2004–2011	Population-based cross-sectional study (L)	8508	Autorefraction	1110 (971)	7444	67 (48–92)	54.5	99.7 (0)	23.0	39.4
Tromsø eye study, Norway	2007–2008	Population-based cohort (L)	6565	Autorefraction	773 (700)	5792	61 (38–87)	55.9	NA (100)	19.4	33.7
TwinsUK, UK	1998–2010	National twin cohort (N)	6245	Autorefraction	161 (61)	6095	55 (16–85)	91.2	98.2 (23.9)	31.4	26.0
Southern Europe											
Thessaloniki eye study, Greece	1999–2005	Cross-sectional population-based study (L)	2259	Subjective	316 (303)	1952	69 (60–94)	44.7	100 (0)	14.2	39.4
Western Europe											
ALIENOR, France	2006–2008	Population-based cohort (L)	951	Autorefraction	333 (318)	618	79 (73–93)	56.6	NA (100)	16.7	53.6
ERF, Netherlands	2002–2005	Family-based cross-sectional study (L)	2708	Subjective	46 (45)	2662	49 (14–87)	55.1	100 (0)	21.2	27.4
Gutenberg health study, Germany	2007–2012	Population-based cohort (L)	14,679	Autorefraction	610 (610)	14,069	54 (35–74)	49.4	NA (100)	31.9	23.9
KORA, Germany	2004–2005	Population-based cohort (L)	3078	Autorefraction	706 (177)	2372	55 (35–84)	50.4	100 (0)	36.1	24.0
Montrachet, France	2009–2013	Population-based cohort (L)	1143	Autorefraction	584 (562)	576	81 (76–92)	57.5	NA (100)	19.1	51.1
Rotterdam Study I, Netherlands	1990–1993	Population-based cohort (L)	6748	Subjective	182 (172)	6566	68 (55–106)	59.3	98.5 (2.0)	16.4	52.3
Rotterdam Study II, Netherlands	2000–2002	Population-based cohort (L)	2689	Subjective	110 (110)	2579	62 (55–99)	54.8	87.8 (0.1)	21.9	45.7
Rotterdam Study III, Netherlands	2005–2008	Population-based cohort (L)	3624	Subjective	94 (74)	3530	56 (46–97)	56.3	NA (100)	32.5	28.8
POLA, France	1995–1997	Population-based cohort (L)	2464	Autorefraction	157 (128)	2315	70 (60–93)	55.8	NA (100)	16.2	53.0

Table 1 continued

Study	Data collection period	Study design	Total with refraction	Refraction method	Exclusions (cataract surgery)	Total included	Median age, years (range)	Gender, % female	Ethnicity, % European (% Unknown)	Crude myopia prevalence, %	Crude hyperopia prevalence, %
Mixed											
EUREYE: Norway, UK, France, Italy, Greece and Estonia	2000–2002	Population based cross-sectional survey in seven cities (L)	4187	Autorefraction or focimetry with subjective refraction	1305 (517)	2882	72 (65–95)	56.7	NA (100)	15.6	59.2
Total cohort	1990–2013		68,350		6404 (4748)	61,946	62	57.6	98.1	25.8	34.4

Myopia ≤ -0.75 diopters (D), hyperopia $\geq$ID, *N* national, *L* local

25–29 years], compared to those in middle to older age [31.2 % (95 % CI 27.5–34.9) in those aged 55–59 years] although hyperopia rates declined after 75 years of age. The prevalence of high hyperopia followed a similar pattern, affecting 1–3 % of younger and 10–13 % of older individuals (Fig. 3c). Across all ages, the prevalence of astigmatism was 27.3 % (95 % CI 22.6–32.1) with an age-standardised estimate of 23.9 % (95 % CI 23.7–24.1). The prevalence of astigmatism remained fairly stable at 15–25 % in young and middle-aged participants [17.0 % (95 % CI 15.1–18.8) in those aged 45–49 years]. However, in participants over 65 years of age, astigmatism became more common [51.1 % (95 % CI 40.4–61.8) in those aged 80–84 years; Fig. 3d].

Age- and gender- specific analyses for myopia, hyperopia and astigmatism are reported in Table 3. There were no significant differences in myopia prevalence between men and women across age strata. However, overall there was a significantly higher prevalence of astigmatism in men ($p = 0.001$), with a mean difference of 3.8 % across all ages, and a significantly higher prevalence of hyperopia in women ($p = 0.04$) with a mean difference of 2.5 % across all ages.

Differences in the myopia prevalence between different European regions, according to the UN European Geoscheme, were examined. Only one cohort contributed to the Southern European division (Thessaloniki Eye Study, Greece), with participants all over the age of 60 years, thus the majority of the studies were in Northern and Western regions. The prevalence of myopia did not differ between Northern and Western countries and followed a similar pattern across all age groups. The single Southern participant cohort appeared to have a higher level of myopia in its older participants when compared to Northern and Western countries, however there were large CIs for these estimates (80–84 year-old myopia prevalence in North 13.6 % (95 % CI 9.3–18.0), West 18.0 % (95 % CI 16.1–21.1) and South 29.1 % (95 % CI 19.1–39.1). Overall there were no significant differences across age strata between the three regions of Europe studied ($p = 0.70$).

Discussion

Meta-analysed data from fifteen population-based adult cohort and cross-sectional studies across Europe indicated age-standardised prevalence of 30.6 % for myopia, 25.2 % for hyperopia and 23.9 % for astigmatism. This meta-analysis usefully incorporates data from across Europe and is not limited to a particular place or age group. The most significant burden of refractive error within Europe was from myopia.

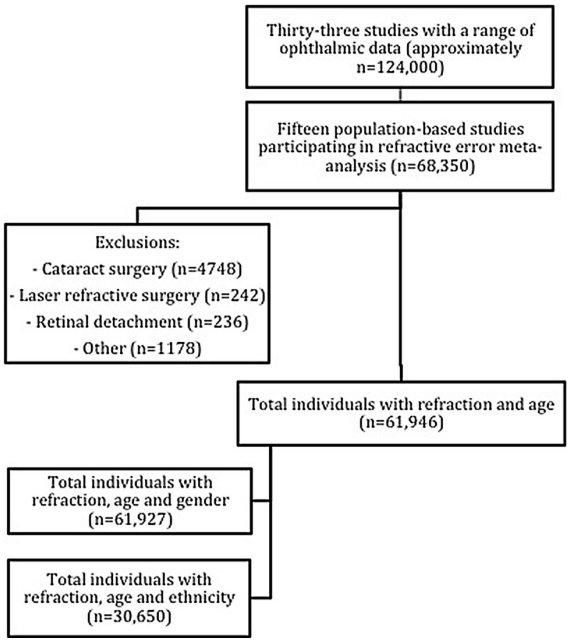

Fig. 1 Flow chart of refractive error meta-analysis within E^3

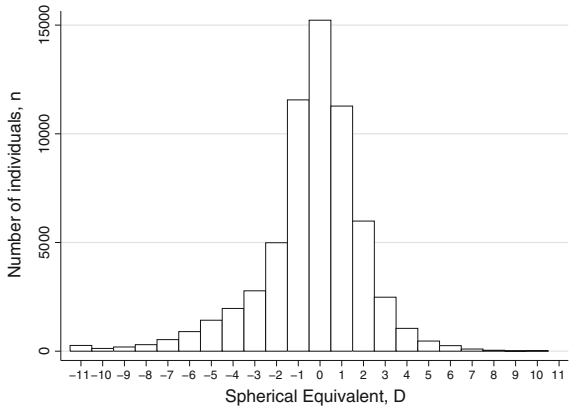

Fig. 2 Distribution of refractive error (*D* diopters)

A clear trend of higher levels of myopia in younger individuals was identified, with a rising prevalence during late teens and 20 s reflecting the known natural history of the condition [16]. The peak prevalence of myopia was identified in the 25–29 years age group (47.2 % (95 % 41.8–52.5). In older individuals, the prevalence of myopia was lower, for example 15.9 % (95 % CI 13.7–18.1) in those aged 65–69 years old. This may reflect the rising prevalence of myopia in younger generations, or the known hyperopic shift in aging [17, 18]. In our aged 75 or over participants, there was an increase in myopia prevalence. While we aimed to exclude those having undergone cataract surgery (and participants with documented cataract in some studies), the rise in myopia likely reflects the development of nuclear cataract, which is known to be associated with a myopic shift as a result of increasing lens power [19]. However, this age-related change in refraction may also occur irrespective of visible lens opacity; in the Beaver Dam Study, a 10-year longitudinal myopic shift (−0.19D, 95 % CI −0.32 to −0.06, $p < 0.001$) was observed in those over 70 years old, even after adjusting for nuclear sclerosis grading [17]. We did not confirm the observation of previous studies of higher myopia prevalence in women [20].

In comparison to previous estimates, the overall burden of myopia in our population appears similar but slightly greater to that of other studies. The 2004 Eye Diseases Prevalence Research Group estimated myopia prevalence at 26.6, 25.4 and 16.4 % for European, North American and Australian sub-analyses respectively [8]. This study included the Beaver Dam Eye Study [21], the Baltimore Eye Survey [22], the Blue Mountains Eye Study [23], the Melbourne Visual Impairment Project [24] and the Rotterdam Study I [9], which was also included in this meta-analysis. In their youngest cohort (40–49 years), 36.8 % of white men and 46.3 % of white women were myopic, similar to our estimates of 42.0 and 39.8 % in 40–44 year-olds, albeit with no gender difference. The US 1999–2004 NHANES examined refractive error variation by age in three ethnicities; the prevalence of myopia in non-Hispanic white participants 20–39 years of age was 35.1 % in men and 42.3 % in women, whilst the prevalence in those ≥60 years was 23.1 % in men and 18.6 % in women [20]. These prevalence rates are again very similar to that found in our data, although we did not find higher levels of myopia in young females. Both comparative estimates are based on a definition of myopia ≤−1D, and are therefore not directly comparable to our study definition of myopia ≤−0.75D, an issue often encountered in refractive error epidemiology where there is a lack of consensus on definitions of refractive error. The adult prevalence of myopia in South-east Asia is of much greater magnitude than that seen in studies of European ancestry [25–28], with remarkably high levels of myopia seen in young individuals [29, 30]. The number of participants in our meta-analysis of Asian origin was very low, precluding meaningful reporting of these estimates.

High myopia prevalence was relatively low in Europe, with an age-standardised estimate of 2.7 % (95 % CI 2.69–2.73). The highest prevalence was observed in younger participants, albeit with wider CIs due to smaller sample size (Table 2). Prevalence in older participants was low, potentially reflective of generational changes, or perhaps exclusion due to the earlier need for cataract surgery in high myopes compared to other refractive groups [31].

Table 2 Prevalence of myopia, hyperopia and astigmatism stratified by age

Age	n	Myopia, % (95 % confidence intervals)				Hyperopia, % (95 % confidence intervals)		Astigmatism, % (95 % confidence intervals)
		All myopia ≤−0.75D (n = 15,845)	Low myopia ≤−0.75 to >−3D (n = 10,034)	Moderate myopia ≤−3 to >−6D (n = 4383)	High myopia ≤−6D (n = 1445)	All hyperopia ≥+1D (n = 21,201)	High hyperopia ≥+3D (n = 4494)	All astigmatism ≥D (n = 15,496)
25–29	339	47.2 (41.8–52.5)	26.5 (21.8–31.2)	14.1 (5.1–23.2)	5.3 (2.9–7.7)	6.4 (3.8–9.0)	1.1 (0.0–2.2)	16.2 (12.3–20.1)
30–34	469	38.3 (22.6–53.9)	25.5 (16.7–34.2)	9.4 (4.2–14.6)	3.2 (1.5–4.9)	5.5 (3.4–7.5)	1.8 (−1.1–4.6)	18.2 (14.3–22.0)
35–39	2354	40.1 (29.2–51.0)	25.8 (15.5–36.0)	10.0 (7.9–12.1)	3.7 (1.3–6.1)	5.8 (3.0–8.6)	1.4 (0.5–2.3)	16.2 (14.5–17.9)
40–44	5552	40.2 (32.0–48.5)	27.5 (19.7–35.3)	9.6 (7.0–12.3)	3.3 (1.8–4.8)	7.9 (6.3–9.5)	2.2 (1.6–2.7)	15.7 (13.2–18.1)
45–49	4108	37.1 (29.4–44.7)	25.1 (18.8–31.4)	9.0 (6.5–11.4)	2.9 (1.8–4.0)	10.3 (7.5–13.2)	2.4 (1.7–3.1)	17.0 (15.1–18.8)
50–54	5684	33.6 (29.6–37.6)	20.9 (18.6–23.2)	9.8 (8.0–11.6)	2.7 (1.4–4.0)	18.0 (15.6–20.4)	3.3 (2.6–3.9)	20.1 (16.3–23.8)
55–59	8294	27.5 (23.5–31.5)	16.6 (14.2–18.9)	8.3 (6.6–9.9)	2.5 (1.9–3.1)	31.2 (27.5–34.9)	5.7 (4.6–6.8)	22.5 (18.2–26.9)
60–64	10,594	21.4 (17.5–25.2)	13.0 (10.9–15.2)	6.0 (4.5–7.4)	2.0 (1.4–2.7)	31.2 (27.5–34.9)	7.5 (6.0–9.0)	25.2 (20.3–30.0)
65–69	9445	15.9 (13.7–18.1)	9.8 (8.4–11.2)	4.7 (3.7–5.7)	1.4 (1.1–1.6)	50.2 (46.1–54.3)	9.7 (8.2–11.1)	28.0 (22.0–34.0)
70–74	7674	13.9 (11.9–15.9)	9.3 (7.8–10.9)	3.4 (2.8–4.0)	1.0 (0.6–1.5)	54.3 (50.4–58.1)	12.8 (9.9–15.7)	33.8 (26.6–41.1)
75–79	4211	15.9 (13.4–18.4)	10.2 (8.5–11.8)	3.9 (2.9–5.0)	1.5 (1.0–1.9)	56.3 (52.1–60.4)	12.8 (9.9–15.7)	44.3 (33.6–55.0)
80–84	2069	17.8 (15.2–20.3)	11.5 (10.1–12.9)	3.8 (2.7–4.9)	1.5 (1.0–2.1)	52.8 (47.9–57.7)	12.0 (9.7–14.3)	51.1 (40.4–61.8)
85–89	683	17.9 (14.0–21.8)	12.4 (9.0–15.8)	3.4 (2.0–4.8)	1.4 (0.4–2.3)	49.2 (42.5–55.9)	13.4 (8.4–18.5)	54.9 (42.9–66.8)
Age standardised prevalence (n = 61,476)		30.60 (30.36–30.85)	19.50 (19.35–19.65)	8.08 (8.01–8.14)	2.71 (2.69–2.73)	25.23 (25.03–25.43)	5.37 (5.33–5.41)	23.86 (23.67–24.05)

D diopters

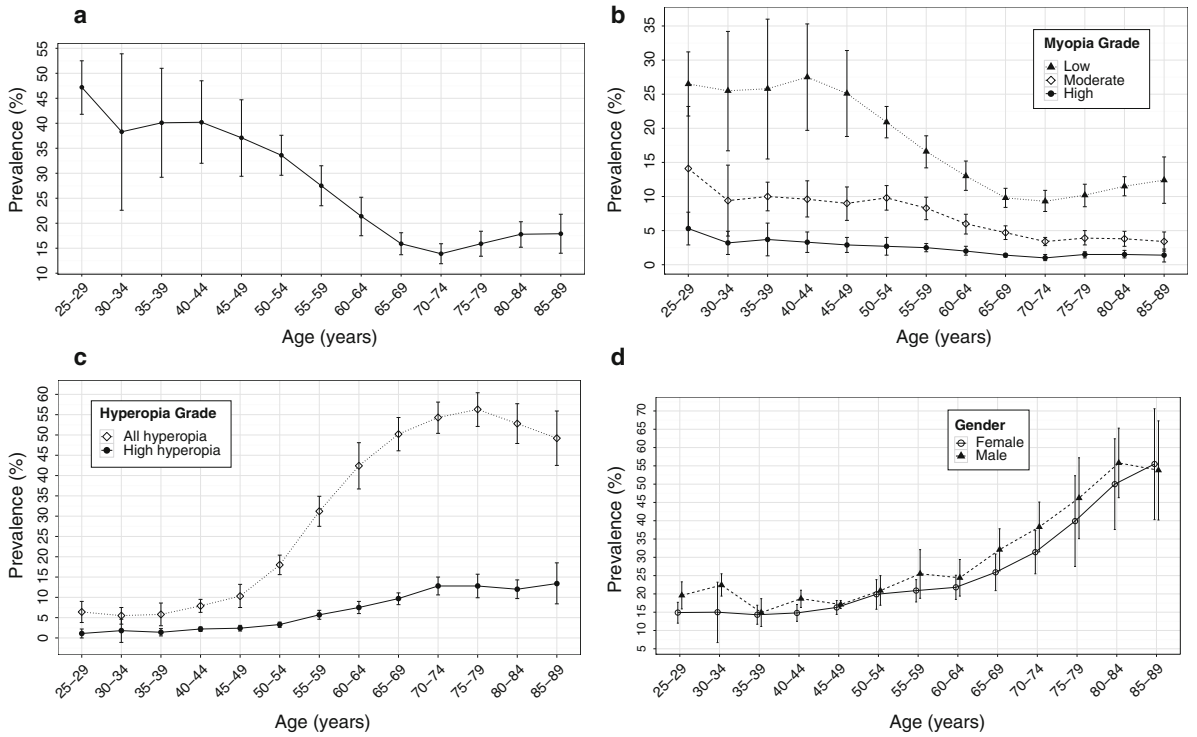

Fig. 3 **a** Prevalence of myopia (SE ≤−0.75D) according to age, with 95 % confidence intervals (*D* diopters). **b** Prevalence of myopia (low myopia SE ≤−0.75 to >−3D, moderate myopia SE ≤−3 to >−6D, high myopia SE ≤−6D) according to age, with 95 % confidence intervals (D, diopters). **c** Prevalence of hyperopia (all hyperopia SE ≥1D, high hyperopia SE ≥3D), according to age, with 95 % confidence intervals (*D* diopters). **d** Prevalence of astigmatism (≥1D) according to age for males and females with 95 % confidence intervals (*D* diopters)

Our greatest high myopia prevalence of 5.9 % (95 % CI 1.3–10.5) in 15–19 year-olds remains much lower than that seen in, for example, urban China where up to 14 % of 17 year-olds are highly myopic [32]. In non-Hispanic White individuals in the NHANES 1999–2004 data, high myopia appeared slightly more common than in our data; for example in those aged 20–29 years-old "severe" myopia was identified in 7.4 %, compared to 2.8 and 5.3 % in those aged 20–24 and 25–29 respectively in this European study. However the NHANES definition of severe myopia (≤−5D) again differs slightly from our definition of high myopia (≤−6D).

Using the same definition of high hyperopia (≥ 3D), our study appeared to have less hyperopia than the Eye Diseases Research Group [8]; for example in 70–74 year-olds 21.3 % of white women and 16.9 % of white men were highly hyperopic compared to just 12.8 % in our European data, which may again reflect a generational or cohort effect.

Astigmatism rates were fairly constant (15–25 %) across cross-sectional age categories, but were higher after the age of 65. This finding has been observed in other studies, together with a shift from with-the-rule to against-the-rule astigmatism [20, 23, 28]. Across all age groups, we identified higher astigmatism prevalence in men, particularly evident in middle to later ages (for example 39.5 % in women and 46.2 % in men aged 70–74). This observation was similar in the older participants of the NHANES 1999–2004 study, where in participants over the age of 60 years the astigmatism prevalence in women was 46.1 % and in men 54.9 % [20].

The major strength of our study is the large sample size contributing to the prevalence estimates, providing a unique opportunity to estimate the burden of refractive error in middle and older aged individuals across Europe. This is beneficial for planning of clinical services and raises awareness, for both clinicians and economists, of the future potential issues of rising myopia levels and associated visual impairment [33]. Refractions were all non-cycloplegic, which is common practice for population-based adult ophthalmic epidemiological studies, thus making this study comparable to previous research [34, 35].

Despite age and gender stratification, significant heterogeneity between studies remained in the meta-

Table 3 Prevalence of myopia, hyperopia and astigmatism stratified by age and gender

Age	n		Myopia, ≤−0.75D (95 % confidence intervals)		Hyperopia, ≥+1D (95 % confidence intervals)		Astigmatism, ≥1D (95 % confidence intervals)	
	Women	Men	Women	Men	Women	Men	Women	Men
25–29	278	59	47.9 (40.0–55.8)	40.2 (22.7–57.8)	6.1 (6.1–6.2)	11.2 (−1.5–23.8)	14.9 (12.0–17.7)	19.6 (15.9–23.3)
30–34	307	160	40.3 (32.6–47.9)	41.7 (10.7–72.7)	4.3 (0.0–8.6)	5.2 (2.9–7.5)	15.0 (6.7–23.2)	22.4 (19.4–25.5)
35–39	1352	1004	40.2 (30.5–49.8)	40.9 (26.2–55.5)	5.6 (3.0–8.1)	6.5 (3.8–9.2)	14.3 (11.7–16.9)	14.9 (11.1–18.7)
40–44	2989	2561	39.8 (31.4–48.2)	42.0 (33.4–50.6)	7.9 (6.6–9.1)	8.4 (7.0–9.8)	14.8 (12.5–17.1)	18.7 (16.3–21.0)
45–49	2258	1849	37.1 (29.8–44.3)	37.1 (25.8–48.3)	11.4 (9.1–13.8)	8.3 (6.6–9.9)	16.3 (14.4–18.2)	17.1 (16.0–18.2)
50–54	3369	2315	34.1 (30.0–38.3)	33.0 (27.7–38.3)	19.1 (17.6–20.7)	17.3 (15.1–19.5)	19.9 (15.8–23.9)	20.9 (16.9–25.0)
55–59	5086	3206	25.8 (21.6–30.1)	29.8 (23.7–35.9)	32.6 (29.8–35.5)	30.6 (25.9–35.2)	20.9 (17.8–23.9)	25.5 (18.8–32.1)
60–64	6364	4226	19.1 (15.9–22.3)	20.7 (17.0–24.4)	43.7 (38.8–48.6)	36.1 (31.9–40.2)	21.8 (18.5–25.1)	24.4 (19.4–29.4)
65–69	5207	4237	14.5 (12.0–17.1)	16.6 (14.2–18.9)	52.3 (48.2–56.4)	48.4 (44.7–52.1)	25.9 (20.9–30.9)	32.1 (26.3–37.8)
70–74	4110	3562	14.2 (12.2–16.2)	14.3 (12.3–16.3)	55.8 (52.2–59.4)	55.0 (51.5–58.5)	31.4 (25.5–37.3)	38.3 (31.5–45.1)
75–79	2290	1920	14.3 (11.5–17.0)	17.7 (14.9–20.5)	58.0 (55.3–60.6)	52.3 (48.0–56.7)	39.9 (35.1–57.2)	46.2 (35.1–57.2)
80–84	1158	911	15.8 (12.8–18.7)	18.7 (16.2–21.3)	57.6 (53.0–62.2)	47.8 (42.6–53.0)	50.0 (37.6–62.4)	55.8 (46.3–65.3)
85–89	419	264	19.6 (13.2–25.9)	16.1 (11.7–20.5)	50.7 (43.5–57.9)	45.0 (37.9–52.0)	55.5 (40.3–70.6)	53.8 (40.2–67.3)
p diff between groups (ANOVA)			0.603		0.042		0.001	

D diopters

analysis. There are inherent differences in the included studies in terms of study design, refraction technique and cohort sampling, together with between country differences in levels of urbanisation, economy, education and climate which may influence refractive error. We were unable to stratify by these factors in this meta-analysis as person-specific data was not available for all studies. This study was mainly comprised of middle and older aged individuals, therefore our estimates of refractive error prevalence carry greater confidence for these ages since they are based on more precise estimates with narrow 95 % CIs. The majority of the studies in this meta-analysis originate from Northern and Western European countries, and therefore our estimates of refractive error are more representative of these European countries. Although our sample includes either national or locally recruited population-based studies, like all epidemiological studies there may be a bias of participants volunteering for an eye examination being more 'health conscious'. We suspect this would have little effect on the prevalence of refractive error, and if anything result a slight underestimation of the prevalence. Finally, refractions were performed over a twenty-year period and, therefore our estimates of prevalence may be subject to error given temporal trends in refractive error prevalence. However, refractions were performed between 2000 and 2010 in thirteen out of the fifteen studies, reducing this variability.

In conclusion, this study estimates refractive error affects just over a half of European adults. Myopia represented the greatest burden, with an estimated 227.2 million people across Europe affected (using the 2010 European population estimates) [36]. Based on study prevalence estimates of high myopia, this also suggests there are 20.1 million people across Europe who are at higher risk of the associated sight threatening complications, such as retinal detachment, that this degree of myopia confers [33].

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Supplementary Information

Details of contributing studies (Appendix 9.3)

3.3 Increasing prevalence of myopia in Europe and the impact of education

This chapter is presented as a published paper and is an exact copy of the following journal publication:

Katie M Williams, Virginie JM Verhoeven, Phillippa Cumberland, Geir Bertelsen, Christian Wolfram, Jugnoo Rahi, Cécile Delcourt, Caroline CW Klaver, Christopher J Hammond (on behalf of the European Eye Epidemiology Consortium (E^3)). Increasing prevalence of myopia in Europe and the impact of education. *Ophthalmology*. 2015 Jul;122(7):1489-97. doi: 10.1016/j.ophtha.2015.03.018.

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The Eye M.D. Association

Increasing Prevalence of Myopia in Europe and the Impact of Education

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Purpose: To investigate whether myopia is becoming more common across Europe and explore whether increasing education levels, an important environmental risk factor for myopia, might explain any temporal trend.

Design: Meta-analysis of population-based, cross-sectional studies from the European Eye Epidemiology (E^3) Consortium.

Participants: The E^3 Consortium is a collaborative network of epidemiological studies of common eye diseases in adults across Europe. Refractive data were available for 61 946 participants from 15 population-based studies performed between 1990 and 2013; participants had a range of median ages from 44 to 78 years.

Methods: Noncycloplegic refraction, year of birth, and highest educational level achieved were obtained for all participants. Myopia was defined as a mean spherical equivalent ≤ -0.75 diopters. A random-effects meta-analysis of age-specific myopia prevalence was performed, with sequential analyses stratified by year of birth and highest level of educational attainment.

Main Outcome Measures: Variation in age-specific myopia prevalence for differing years of birth and educational level.

Results: There was a significant cohort effect for increasing myopia prevalence across more recent birth decades; age-standardized myopia prevalence increased from 17.8% (95% confidence interval [CI], 17.6–18.1) to 23.5% (95% CI, 23.2–23.7) in those born between 1910 and 1939 compared with 1940 and 1979 ($P = 0.03$). Education was significantly associated with myopia; for those completing primary, secondary, and higher education, the age-standardized prevalences were 25.4% (CI, 25.0–25.8), 29.1% (CI, 28.8–29.5), and 36.6% (CI, 36.1–37.2), respectively. Although more recent birth cohorts were more educated, this did not fully explain the cohort effect. Compared with the reference risk of participants born in the 1920s with only primary education, higher education or being born in the 1960s doubled the myopia prevalence ratio–2.43 (CI, 1.26–4.17) and 2.62 (CI, 1.31–5.00), respectively—whereas individuals born in the 1960s and completing higher education had approximately 4 times the reference risk: a prevalence ratio of 3.76 (CI, 2.21–6.57).

Conclusions: Myopia is becoming more common in Europe; although education levels have increased and are associated with myopia, higher education seems to be an additive rather than explanatory factor. Increasing levels of myopia carry significant clinical and economic implications, with more people at risk of the sight-threatening complications associated with high myopia. *Ophthalmology 2015;122:1489-1497 © 2015 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

Supplemental material is available at www.aaojournal.org.

Myopia (near-sightedness) occurs when a distant object's image is formed anterior to the retinal plane, most commonly as a result of an increased axial length. This results in blurred distant vision and, unlike hyperopia, requires refractive correction at all ages and severity for clear focus. Myopia is already the most common eye condition worldwide, but the prevalence is significantly increasing, especially in Southeast Asia.[1–3] In Europe, Australia, and the United States, the

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Ophthalmology Volume 122, Number 7, July 2015

prevalence of myopia seems to be lower[4,5]; however, there is evidence of an increasing prevalence in the United States and elsewhere,[6–8] particularly among young adults.[9] This is of concern because myopia, even when appropriately corrected, is associated with an increased risk of sight-threatening diseases, such as myopic maculopathy, retinal detachment, glaucoma, and cataract.[10] Myopic maculopathy is currently untreatable and already contributes to visual impairment in working-age adults.[11] Increasing myopia levels in Europe carry implications for public health policy in both the provision of clinical services and the economic sequelae from the resulting visual impairment among the working population.

Myopia is a highly heritable trait,[12,13] and to date a number of genetic polymorphisms have been associated with refractive error, albeit explaining only a small proportion of this heritability.[14,15] Environmental factors play a key role in myopia development and must explain the recent changes in prevalence.[16] Myopia has been associated with education, near work, urbanization, prenatal factors, socioeconomic status, cognitive ability, season of birth, light, and time spent outdoors.[2,16–25] One of the strongest and most replicated risk factors is educational attainment,[16,26] and there is some evidence of interaction between genetic factors and education influencing the risk of myopia.[27] The increased levels of higher education over the 20th century[28] might be a causative factor, or marker of a causative factor, for increasing myopia prevalence.

The aims of this study are to identify whether myopia is becoming more common across Europe and to examine whether increasing levels of education explain any temporal trend, using data from more than 60 000 participants from the European Eye Epidemiology (E^3) Consortium.

Methods

Study Population

The E^3 consortium is a collaborative initiative to share and meta-analyze epidemiologic data on common eye diseases across Europe. Thirty-three studies are currently part of the consortium, and a range of ophthalmic data are available on approximately 124 000 individuals from population-based and case-control cohorts. All studies adhere to the tenets of the Declaration of Helsinki, and relevant local ethical committee approvals with specific study consent were obtained.

Refractive error measurements from 68 350 adults within the 15 E^3 population-based studies that had data on refractive error were included. These included population-based cross-sectional or cohort studies, with 2 studies recruiting participants nationally and 13 studies recruiting from a local population. Further details on each study are provided in Table 1 and the Supplementary information (available at www.aaojournal.org). Exclusion criteria included subjects who had cataract or refractive surgery, retinal detachment, or other conditions, such as keratoconus, which might influence refraction (n = 6404). Data on age at refraction and birth year were available for 61 946 individuals, with information on education level for 60 125 subjects. Participants were mainly middle to late age; 98% were of European descent (where ethnicity was known), predominantly from Northern and Western Europe; and refractive examinations were performed from 1990 to 2013 (Table 1).

Study Variables

Noncycloplegic refractions were performed on all individuals using subjective refraction, autorefraction, or a combination of focimetry with subjective refraction. Spherical equivalent was calculated using the standard formula (spherical equivalent = sphere + [cylinder/2]). Myopia was defined as ≤ -0.75 diopters. Myopia prevalence by age was calculated, using 5- and 10-year age bands from $\geq$15 years to $\geq$90 years. To study the impact of education on myopia, given the variation in educational systems across Europe, we established a simplified 3-tier level of education across all cohorts. Primary education was defined as those leaving school before 16 years of age, secondary education was defined as those leaving education up to the age of 19 years, and higher education was defined as those leaving education at or after the age of 20 years. Those aged younger than 20 years at the time of refraction (and therefore unable to have reached the highest education tier) were excluded from this analysis to avoid misclassification bias.

We investigated the evidence for a cohort effect on increasing myopia prevalence by observing variations in myopia prevalence within defined age bands. These analyses are focused on the age range constituting the majority of our cohort (40–80 years of age, birth year 1910–1979, n = 56 088), meaning the youngest and oldest participants, for whom we had no comparative birth cohort, were not considered. Prevalence between different birth cohorts was examined, initially using decade bins (1910–1970) and subsequently in 2 birth cohort groups divided by the median birth decade (1940–1949). Finally we examined the influence of education by examining the myopia prevalence between birth cohorts with the additional stratification of educational status.

Statistical Analysis

Study-specific summary data for myopia prevalence were obtained and combined in a random-effect meta-analysis stratified by age. A random-effects model was chosen over a fixed-effects model to allow for expected heterogeneity between studies as a result of varying study design. Age was standardized with demographic distribution adjustments to age-specific estimates according to the European Standard Population 2010.[29] Evidence for the presence of a cohort effect was investigated using random-effect meta-analyses of myopia prevalence stratified by age and birth year, and subsequently age, birth year, and educational level. Differences between estimates of myopia prevalence were evaluated using the analysis of variance test, proportion z tests, and prevalence ratios (relative difference in prevalence against a defined baseline). Differences were considered significant at $P < 0.05$.

Statistical analysis was performed using Stata statistical software version 13.1 (StataCorp LP, College Station, TX). Graphical outputs[30] were obtained using Stata, Origin version 9.0 (OriginLab Corp, Northampton, MA), or ggplot2(30) in R software (R Foundation for Statistical Computing, Vienna, Austria; available at http://www.R-project.org).

Results

In this meta-analysis of 61 946 adults, the overall myopia prevalence was 24.3% (95% confidence interval [CI], 20.1–28.5), with an age-standardized prevalence in Europe of 30.6% (95% CI, 30.3–30.8). Age-stratified analyses[31] revealed a high prevalence in young adults (47.2% [95% CI, 41.8–52.5] in those aged 25–29 years), which was almost double the prevalence in those of middle to older age (27.5% [95% CI, 23.5–31.5] in those aged 55–59 years). There were no significant differences in the myopia prevalence by gender.[31]

Table 1. Description of the 15 European Eye Epidemiology Consortium Studies Included in this Meta-Analysis of Refractive Error

Study	Data Collection Period	Study Design	Total Participants with Refraction	Refraction Method	Exclusions* (Cataract Surgery)	Total Participants Included	Median Age, yrs (Range)	Gender, % Female	Ethnicity, % European (% Unknown)	Higher Education, %	Crude Myopia Prevalence, %
Northern Europe											
1958 British Birth Cohort, UK	2002–2003	Population-based birth cohort (N)	2502	Autorefraction	7 (0)	2495	44 (44–46)	51.7	98.0 (9.2)	29.9	48.7
EPIC-Norfolk, UK	2004–2011	Population-based cross-sectional study (L)	8508	Autorefraction	1110 (971)	7444	67 (48–92)	54.5	99.7 (0)	17.9	23.0
Tromsø Eye Study, Norway	2007–2008	Population-based cohort (L)	6565	Autorefraction	773 (700)	5792	61 (38–87)	55.9	NA (100)	32.5	19.4
TwinsUK, UK	1998–2010	National twin cohort (N)	6245	Autorefraction	161 (61)	6095	55 (16–85)	91.2	98.2 (23.9)	22.3	31.4
Southern Europe											
Thessaloniki Eye Study, Greece	1999–2005	Cross-sectional population-based study (L)	2259	Subjective	316 (303)	1952	69 (60–94)	44.7	100 (0)	Unknown	14.2
Western Europe											
ALIENOR, France	2006–2008	Population-based cohort (L)	951	Autorefraction	333 (318)	618	79 (73–93)	56.6	NA (100)	20.0	16.7
ERF, Netherlands	2002–2005	Family-based cross-sectional study (L)	2708	Subjective	46 (45)	2662	49 (14–87)	55.1	100 (0)	16.9	21.2
Gutenberg Health Study, Germany	2007–2012	Population-based cohort (L)	14 679	Autorefraction	610 (610)	14 069	54 (35–74)	49.4	NA (100)	37.6	31.9
KORA, Germany	2004–2005	Population-based cohort (L)	3078	Autorefraction	706 (177)	2372	55 (35–84)	50.4	100 (0)	14.7	36.1
Montrachet, France	2009–2013	Population-based cohort (L)	1143	Autorefraction	584 (562)	576	81 (76–92)	57.5	NA (100)	Unknown	19.1
Rotterdam Study I, Netherlands	1990–1993	Population-based cohort (L)	6748	Subjective	182 (172)	6566	68 (55–106)	59.3	98.5 (2.0)	11.6	16.4
Rotterdam Study II, Netherlands	2000–2002	Population-based cohort (L)	2689	Subjective	110 (110)	2579	62 (55–99)	54.8	87.8 (0.1)	22.3	21.9
Rotterdam Study III, Netherlands	2005–2008	Population-based cohort (L)	3624	Subjective	94 (74)	3530	56 (46–97)	56.3	NA (100)	31.4	32.5
POLA, France	1995–1997	Population-based cohort (L)	2464	Autorefraction	157 (128)	2315	70 (60–93)	55.8	NA (100)	7.3	16.2
Mixed											
EUREYE: Norway, UK, France, Italy, Greece, and Estonia	2000–2002	Population-based cross-sectional survey in 7 cities (L)	4187	Autorefraction or focimetry with subjective refraction	1305 (517)	2882	72 (65–95)	56.7	NA (100)	30.0	15.6
Total cohort	1990–2013		68 350		6404 (4748)	61 946	62	57.6	98.1	36.0	25.8

ALIENOR = Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes Study; EPIC = European Prospective Investigation into Cancer; ERF = Erasmus Rucphen Family Study; EUREYE = European Eye Study; KORA = Kooperative Gesundheitsforschung in der Region Augsburg; L = local; N = national; NA = not available; POLA = Pathologies Oculaires Liées à l'Age Study. Myopia classified in those with refraction ≤−0.75 diopters.
*Exclusions = cataract surgery, refractive surgery, retinal detachment or other conditions affecting refraction.

Ophthalmology Volume 122, Number 7, July 2015

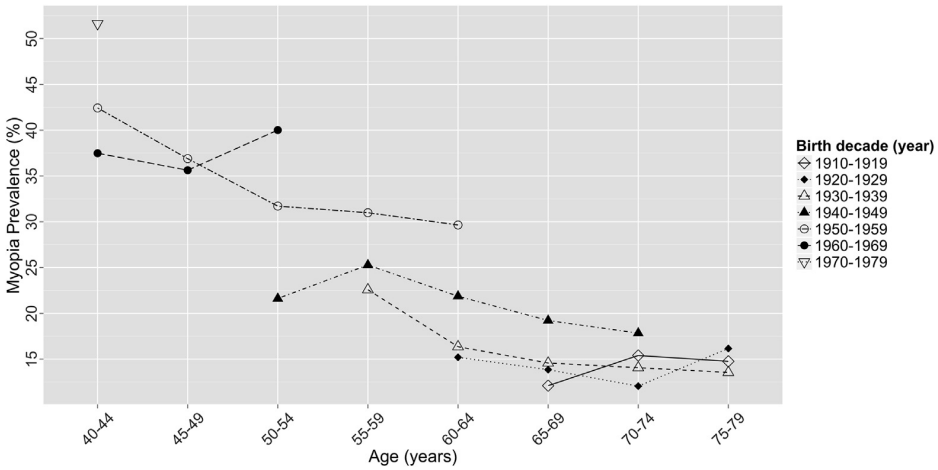

Figure 1. Prevalence of myopia (spherical equivalent ≤−0.75 diopters) against age stratified by decade of birth. Individuals aged 40 to 79 years included.

Cohort Effect for Increasing Myopia Prevalence

There was a trend of higher myopia prevalence with more recent birth decade across all age groups (Fig 1), although sample sizes for some point estimates were low, resulting in wide CIs (Table 2, available at www.aaojournal.org).

We examined the prevalence of myopia in 2 birth cohort groups (divided by the median birth decade): those born between 1910 and 1939 (n = 22 660) and those born between 1940 and 1989 (n = 33 428) (Fig 2). Myopia prevalence in a variance model was significantly higher in the more recent birth cohort group (P = 0.03). Age-standardized myopia prevalence over a comparable age range of 50 to 79 years increased from 17.8% (95% CI, 17.6−18.1) in those born in 1910−1939 to 23.5% (95% CI, 23.2−23.7) in those born in 1940−1979. In age-specific analyses, the prevalence of myopia in those aged 50 to 59 years (at the time of their refraction) was 22.5% (95% CI, 20.2−24.9) in those born before 1940, compared with 29.2% (95% CI, 25.3−33.0) in those born after 1940 (P = 0.004). A similar significant increase of 15.3% (95% CI, 13.4−17.3) to 21.2% (95% CI, 18.6−23.8) was observed in those aged 60 to 69 years (P < 0.001).

Influence of Education on Myopia Risk and the Cohort Effect

The association between education and myopia was investigated in the 13 studies from which these data were available (n = 60 125 participants). Educational level was significantly associated with myopia prevalence across all age strata (P < 0.0001). Overall, the age-standardized myopia prevalence for those completing primary, secondary, and higher education was 25.4% (95% CI, 25.0−25.8), 29.1% (95% CI, 28.8−29.5), and 36.6% (95% CI, 36.1−37.2), respectively. In those aged 35 to 84 years, the majority of study subjects, myopia prevalence in participants with higher education was approximately double those with primary education (Fig 3). For example, in subjects aged 45 to 49 years when tested, the myopia prevalence was 26.3% (95% CI, 20.1−32.5) compared

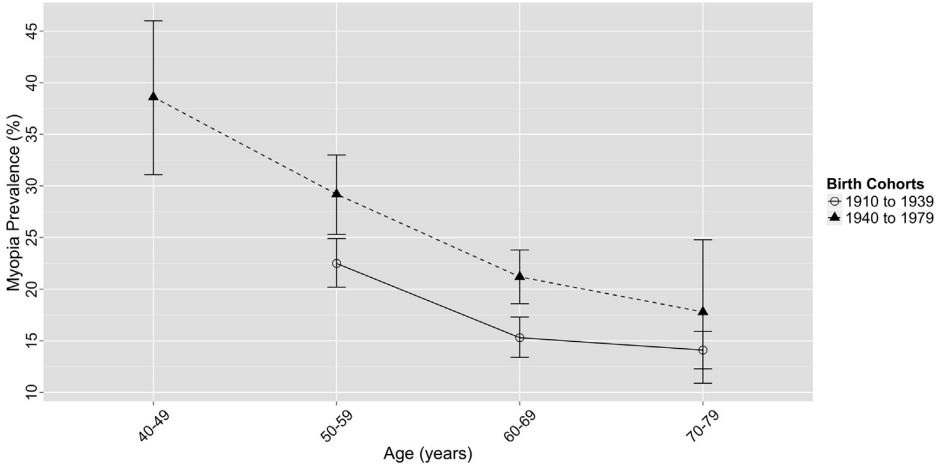

Figure 2. Prevalence of myopia (spherical equivalent ≤−0.75 diopters) as a function of age for 2 birth cohorts (1910−1939, 1940−1979) with 95% confidence intervals.

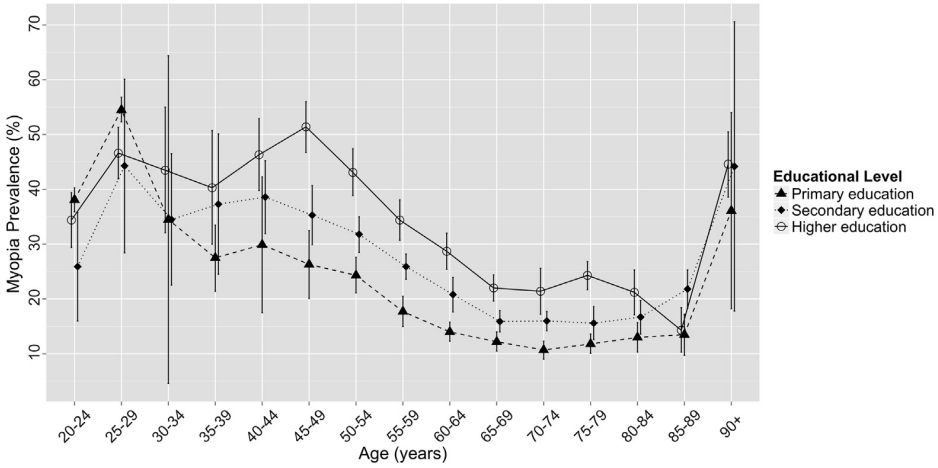

Figure 3. Prevalence of myopia (spherical equivalent ≤−0.75 diopters) with 95% confidence interval stratified by highest educational level achieved: primary education, leaving education at age <16 years; secondary education, leaving school at age ≤19 years; higher education, leaving school at age ≥20 years.

with 51.4% (95% CI, 46.7−56.0) for those with primary and higher education, respectively, and in those aged 60 to 64 years, myopia prevalence was 14.0% (95% CI, 12.3−15.8) compared with 28.7% (95% CI, 25.4−32.0) for those with primary and higher education, respectively. The trends observed are less clear in younger subjects (<35 years) in Figure 3, most likely because of small sample sizes (n = 216 aged 20−25 years, n = 336 aged 25−30 years), which are further stratified by education level with corresponding wide CIs.

Levels of education throughout Europe have increased in the past 90 years (Fig 4). The proportion of individuals progressing to higher education increased from 4% of those born in the 1900s to 16% in the 1920s, 20% in the 1940s, 33% in the 1960s, and approximately 61% in the 1980s.

However, although those born more recently were more likely to have achieved a higher educational level, this alone did not explain the cohort effect of increasing myopia. As shown in Figure 5, for individuals aged 45 to 65 years (age range selected for minimal age-related myopia variance and large available sample size), the increase in myopia prevalence with a more recent birth decade was observed across all educational groups. This was most pronounced for participants achieving only a primary education, in whom myopia prevalence increased from 10.7% (95% CI, 7.6−13.8) to 28.1% (95% CI, 18.1−38.0) between birth decades 1920 to 1929 and 1960 to 1969 ($P = 0.001$). The corresponding increase in myopia in those with higher education was from 26.0% (95% CI, 17.4−34.6) to 40.2% (95% CI, 30.5−50.0) ($P = 0.03$). Compared with the reference risk of participants with primary education and born in the 1920s, the myopia prevalence ratio for those achieving a higher education was 2.43 (95% CI, 1.26−4.17) and for those born in the 1960s was 2.62 (95% CI, 1.31−5.00). Individuals born in the 1960s and completing higher education had approximately 4 times the baseline risk, with a prevalence ratio of 3.76 (95% CI, 2.21−6.57). Thus, the individual associations of educational level and birth cohort had an additive effect on myopia prevalence.

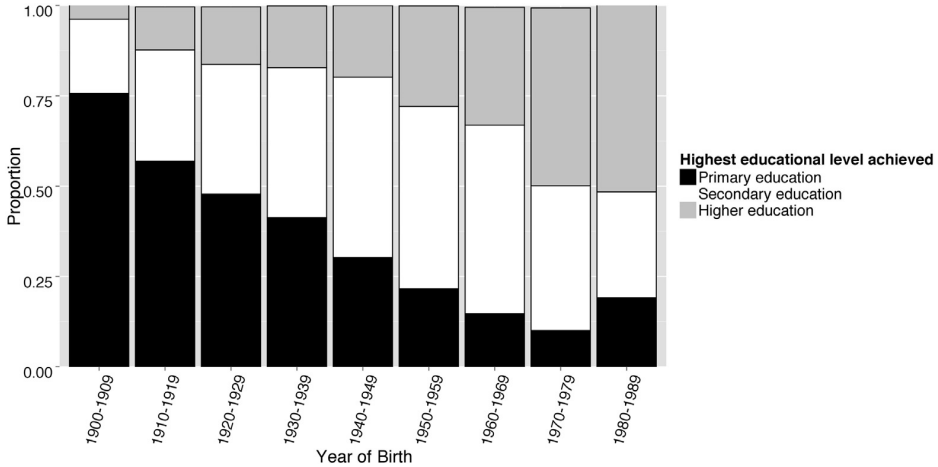

Figure 4. Distribution of highest educational level achieved, stratified by year of birth (1900−1989): primary education, leaving education at age <16 years; secondary education, leaving school at age ≤19 years; higher education, leaving school at age ≥20 years.

Ophthalmology Volume 122, Number 7, July 2015

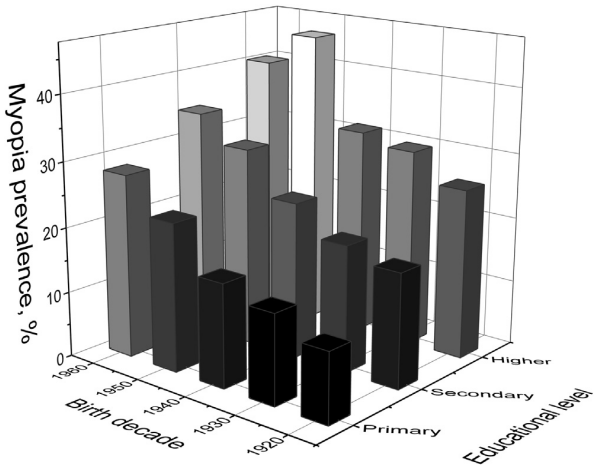

Figure 5. Myopia prevalence (spherical equivalent ≤ -0.75 diopters) by birth cohort and educational level in individuals aged 45 to 65 years: primary education, leaving education at age <16 years; secondary education, leaving school at age ≤19 years; higher education, leaving school at age ≥20 years.

Discussion

Our study provides the first evidence that myopia is becoming more common across Western and Northern Europe, with a clear trend of higher myopia prevalence in participants with a more recent birth year (Fig 1). This is similar to the increase reported in North America and, albeit to a lesser extent, Southeast Asian populations.[6,7,32,33] Evidence of increasing myopia prevalence carries clinical and economic implications. The increased requirement for detection and treatment of myopia, entailing glasses, contact lenses, or more recently laser refractive surgery, has significant implications for clinical optometric and ophthalmic service provision, and the health care system. Additional ophthalmic services will be needed for treatable sight-threatening complications, such as retinal detachment, glaucoma, and cataract.[10,34] The increasing prevalence of myopia also implies that untreatable complications, such as myopic maculopathy, most commonly seen in high myopia, will become more common. This will result in more visual impairment in middle- to older-aged individuals, including a proportion of the working-age population, with consequent economic implications.

Myopia has been strongly associated with education,[2,21,24,35] and we explored this using a simple 3-tier classification of educational level. Increasing educational level had a strong effect, with myopia twice as common in those achieving a higher education compared with participants leaving school before 16 years of age. There was a clear trend of increasing prevalence of myopia across the tiers of education level, suggesting a potential additive effect of years of education. This interesting association may reflect a number of factors: greater near work activities with more education and less time in outdoor light, shared genetic factors underlying myopia and intelligence, or factors related to educational opportunity, such as socioeconomic status or maternal nutrition. These associations have been explored in younger cohorts,[18–21,36,37] although causal pathways are yet to be fully understood.

Reasons for the observed cohort effect are clearly multifactorial, and education is an obvious possible explanation; in our data, only 12% of participants born in the 1920s went on to higher education, compared with 33% born in the 1960s. This educational expansion has been observed across Europe in both men and women, with a sharp trajectory toward mass higher education after World War II.[28,38] In addition to the disruption of education and economic consequences of World War II, adverse health outcomes have been reported in young people growing up at that time, notably diabetes, depression, and heart disease.[39] Although there is no known direct link between these health issues and myopia, the deprivation may have affected eye growth and resulting refraction. Certainly there was an increase in myopia in subjects born after 1950, but it is difficult to be certain what aspect of the seismic changes in Europe after the war might be responsible.

Although the younger generations were more educated, we found a clear increase in the prevalence of myopia across the birth cohorts within each educational stratum, as well as the additive effect of educational status. Therefore, increasing levels of myopia were not explained by education alone, and a more recent birth year and higher educational level had an additive effect on myopia risk. Our simple 3-tier education stratification may be subject to residual confounding from variation in educational practices, and it may be these, rather than changes in education level, that are contributing to the observed cohort effect. In the latter half of the last century, there was increasing use of computers, increasing length of the educational day with increased after-school tuition, and less outdoor play as a result of reduced recess time.[35]

Study Limitations

The E^3 consortium has provided a large data set to meta-analyses' temporal trends and educational associations for myopia prevalence across Europe. Limitations to this consortium meta-analysis include heterogeneity between studies. Contributing studies inherently differed in study design and cohort sampling. In acknowledgment of this heterogeneity, we performed a random-effect rather than a fixed-effect meta-analysis, assuming no fixed effect between studies. There are also differences between European countries in terms of urbanization, economy, social class, education, and lifestyle, which are known to influence myopia. Data on these variables at an individual or study-specific level were not uniformly available, and data often were collected from middle-aged and older participants, so retrospective collection of potential contributing factors such as outdoor exposure, amount of reading, and area of residence during the critical first 20 years of refractive error development would be impossible. In addition, potential multicollinearity of these likely highly correlated factors (e.g., reading and education) would make assessment of separate effects difficult. In an attempt to reduce heterogeneity arising from these associated

factors, we stratified the random-effects meta-analysis by age and educational level (both significantly associated with myopia). Applicability of our findings is greatest for middle- to older-aged individuals and for those from Northern and Western European countries, given the sampled ages and the location of the E^3 studies (Table 1), although ultimately the degree to which these studies are representative of the underlying population is unknown.

Further limitations include the crude nature in which education was classed, which as previously acknowledged may result in residual confounding. In addition, education status was collected retrospectively and therefore prone to recall error, possibly heightened in older participants. Refractions were all noncycloplegic, although this is reasonable given the age of participants.[40,41] Finally, these data are not longitudinal, so we have not examined reasons for the lower prevalence with age within birth decades, although the cohort effect we identified may be part of this explanation. Other reasons include the well-known hyperopic shift with age and could include other factors, such as censoring with age if myopic subjects receive earlier cataract surgery.

In conclusion, the prevalence of myopia is increasing in Europe, a finding that is not fully explained by increasing education levels despite higher educational achievement being associated with myopia and becoming more widespread in Europe. The changes in prevalence are similar to those observed in North America, although they remain far less than those identified in Southeast Asia, possibly because of differing intensity of education from an early age.[1,6,35] High levels of myopia were detected in the younger adults with a more recent birth year, of whom approximately half were affected. This has significant implications for the future; increasing myopia prevalence, and specifically high levels in younger individuals, will potentially result in an increasing burden of associated visual impairment in the future.

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Abbreviations and Acronyms:
CI = confidence interval; **D** = diopters; **E^3** = European Eye Epidemiology.

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Supplementary Table 2

	Birth decade						
Age	**1910-1919**	**1920-1929**	**1930-1939**	**1940-1949**	**1950-1959**	**1960-1969**	**1970-1979**
40-44					42.4 (21.6-63.2)	37.5 (25.7-49.3)	51.6 (22.4-80.9)
45-49				*	36.9 (27.6-46.2)	35.6 (28.5-42.8)	
50-54				21.6 (14.2-29.1)	31.7 (27.0-36.5)	40.0 (33.0-47.0)	
55-59			22.6 (20.2-25.0)	25.3 (21.9-28.7)	31.0 (28.1-33.9)		
60-64		15.2 (12.7-17.7)	16.4 (13.2-19.5)	21.9 (18.8-25.0)	29.7 (18.9-40.4)		
65-69	12.1 (9.5-14.7)	13.9 (11.2-16.5)	14.6 (12.0-17.1)	19.2 (16.5-21.9)			
70-74	15.4 (13.0-17.8)	12.1 (8.8-15.3)	14.1 (11.4-16.7)	17.8 (10.9-24.8)			
75-79	14.8 (10.9-18.6)	16.2 (13.6-18.7)	13.6 (10.4-16.7)				

Table 2 Prevalence of myopia (Spherical Equivalent ≤ -0.75 Diopters) against birth year stratified by age. Individuals aged 40 to 79 included. (* = meta-analysis not possible due to single contributing prevalence estimate)

Supplementary Information

Details of contributing studies (Appendix 9.3)

3.4 Age of myopia onset in a British population-based twin cohort

This chapter is presented as a published paper and is an exact copy of the following journal publication:

Katie M Williams, Pirro G Hysi, Abhishek Nag, Ekaterina Yonova-Doing, Cristina Venturini, Christopher J Hammond. Age of myopia onset in a British population-based twin cohort. *Ophthalmic Physiol Opt*. 2013 May;33(3):339-45. doi: 10.1111/opo.12042. Epub 2013 Mar 20

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Age of myopia onset in a British population-based twin cohort

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Keywords: age of onset, myopia, prevalence, refractive error

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Abstract

Purpose: School-age myopia is becoming more common in Asia and North America; data from the United Kingdom has suggested a significant amount of myopia develops after the age of 17 years. Age of spectacle wear has been used as a proxy of myopia severity in a recent large genome-wide association study. The purpose of this study was to examine the age of onset of spectacle wear in a large British twin cohort, to examine the reliability and reproducibility of self-reported age of onset as a proxy measure of myopia severity, and to see if there is evidence in the UK of a rising prevalence of myopia.

Methods: Non-cycloplegic autorefraction was performed on over 6000 subjects from the TwinsUK cohort, a large, well-characterized volunteer cohort of British, predominantly Caucasian female twins, between 1998 and 2010. Questionnaires asking age of first spectacle wear were conducted in 2003 and 2008. Myopia was defined as worse than or equal to −1.00 Dioptres, and adult onset myopia as occurring on or after the age of 17 years.

Results: Autorefractive data was available on 6097 participants at a mean age of 53 years. The mean S.E. was −0.36 D (S.D. 2.67, range −25.13 to +9.38). 1705 subjects (28%) were myopic with a mean refractive error of −3.54 (S.D. 2.51, range −25.13 to −1.00) and the median age of first glasses wear was 15 years (mean 18.4 years, S.D. 12.24, range 0–74). Of those who provided an age at which they first wore glasses in both questionnaire sources (n = 628), there was median difference in response of 0 years (S.D. 7.18, mean 0.7, maximum 53). A statistically significant cohort effect for increased myopia prevalence across a range of age groups between 1998–1999 and 2008–2010 was identified, with myopia prevalence increasing from 27% to 34% in those aged 50–54 and from 16% to 32% in those aged 55–59.

Conclusions: Almost half the myopes in this UK-based population wore glasses after the age of 17; further research into adult-onset myopia is required. Although self-reported age of glasses is reproducible and reflects severity, it only explains approximately 15% of the variance of spherical equivalent, so is a rough proxy of refractive error, but still may be useful in large-scale population studies without access to refraction. We have demonstrated a significant cohort effect for increased myopia prevalence in the UK population over a 10-year period.

Introduction

Myopia is the most common ocular condition and affects over a third of the UK adult population.[1,2] The incidence is increasing in western, urbanized countries; myopia prevalence increased from 25% to 41% over a 30 year period in the United States[3] and recent data from urban East Asia shows the condition is reaching epidemic proportions with

up to 50% of 9 year-olds and over 95% of University freshmen myopic.[4,5] Myopia is associated with a number of sight threatening complications. Myopic macular degeneration, which generally develops in adulthood in those with high myopia, is an important cause of blindness in working age adults (14.2% in a recent Scandinavian study[6]). The World Health Organization (WHO) has identified 153 million people visually impaired due to uncorrected refractive errors. The identification and treatment of myopia is now a WHO priority within their initiative to eliminate avoidable blindness.[7]

Myopia is variably defined as a refractive error equal to or worse than −0.25 to −1.00 dioptres (D),[8,9] and can be graded according to severity and age of onset. The severity of myopia is generally categorized as low (−0.50 to −2.99 D), moderate (−3.00 to −5.99 D) or severe (worse than or equal to −6.00). [1,10] There have been various classifications of age of onset of myopia.[11] More recently myopia onset has been divided into adult and what is variably termed childhood, early or school onset myopia.[12] Most of the research on environmental factors such as close work, lack of outdoor light, education and socioeconomic status which play a key role on myopia risk have been performed in this age group.[13–16] Early onset of myopia usually leads to higher final levels of myopia and a higher risk of potential associated complications; in a UK study spectacle wear for myopia before the age of 9 years predicted high myopia in adulthood ($\geq$ −6.00 D) with 73% specificity and 80% sensitivity.[17]

A significant but variable proportion of myopia has been found to develop in early adulthood. The most substantive evidence of the age of myopia onset in the UK is from the analysis of the 1958 British Birth Cohort, a nationally representative sample who were measured at 44/45 years of age. This suggested that the majority of myopes, almost three quarters, had good uncorrected visual acuity at the age of 16.[1] Longitudinal changes in refraction were studied in British clinical microscopists[18] where 39% developed adult-onset myopia which biometric analysis showed was predominantly due to vitreous chamber elongation, as in school-onset myopia.[19,20] This raises not only the question of what factors may be involved in adult-onset myopia, but also of possible misclassification of subjects into 'non-myopes' if they are assigned at too young an age.

The commercial genomic company, 23andMe (https://www.23andme.com), recently reported a genome wide analysis study (GWAS) including 23 000 myopes, which was performed without any refractive data.[21] Myopia was defined in anyone who self-reported the diagnosis and severity was inferred from the self-reported age at which glasses were first required. This methodology and data source is in strong contrast to the expectations of most visual researchers who would generally consider careful quantitative refraction fundamental to any research into myopia. However their replication of previously reported GWAS results,[22,23] and international meta-analysis results[24] was striking.

The aim of this study was to examine the age of onset of myopia in a large cohort of British adult twins and to assess the validity of using self-reported age at which glasses were first worn as a proxy for myopia onset and severity. The study cohort included individuals examined over a 10-year period with a 70-year range in birth years, thus enabling investigation of a potential cohort effect.

Methods

Subjects volunteered through media campaigns to be on the TwinsUK Adult Twin Registry at St Thomas' Hospital, London (83% female, predominantly middle-aged and older).[25] Registry subjects were invited to attend the hospital for a visit which involved collection of various phenotypes and venepuncture for DNA extraction. Included in the phenotyping was an eye examination where refractive error was measured using non-cycloplegic autorefraction (ARM-10 autorefractor). Phenotyping was performed between 1998 and 2010, during a number of TwinsUK eye studies. These included a study of genes and environment in refractive error conducted between 1998 and 1999,[26] the TwinsUK study 2004–2006, a study of the heritability of macular pigment in 2004,[27] and the Healthy Aging in Twins Study (HATS) between 2007 and 2010.[28,29] Refraction was performed on more than one occasion in some individuals (n = 1491); in that scenario the earliest refraction obtained was used.

Age of myopia onset was inferred from reported age at which the individual started wearing glasses. Age at onset of glasses for distance (and/or reading) was asked in a TwinsUK questionnaire conducted between 2002 and 2003 (Appendix S1). For twins who did not provide an answer in this questionnaire, the 'HATS' questionnaire conducted between 2007 and 2010 where twins were asked at what age they started wearing glasses, was used[29] (Appendix S2). If myopes gave differing ages in the two questionnaires the earlier questionnaire was used, unless the more recent questionnaire was less than the age given in the older questionnaire, provided the reported age at which reading glasses were required was either the same or greater than the age for distance glasses. Adult onset myopia was defined as myopia developing at or after the age of 17 years, to allow direct comparison with the 1958 British Birth Cohort.[1]

Subjects were excluded if they gave a history of cataract surgery, laser refractive surgery, or retinal detachment. Spherical equivalent (S.E.) was recorded in the standard manner as the sum of the spherical power and half the

cylindrical power in dioptres. The mean S.E. for both eyes was calculated for each individual, and where data were available for only one eye, this was used as the SE for the subject. Myopia was defined as S.E. ≤ -1.00 D (i.e. more than or equal to 1 D of myopia). This definition of myopia was used to allow comparison with the Eye Disease Prevalence Research Group publication on the prevalence of myopia in the United States, Australia and Western Europe.[8] High myopia was defined as S.E. ≤ -6 D, moderate myopia as -5.99 D to -3 D, and low myopia as -2.99 D to -1.00 D.

For all studies, adherence to the Declaration of Helsinki was maintained, full informed consent was obtained, and the Local Research Ethics Committee reviewed protocols. Data handling and statistical analysis was performed using STATA software (STATA version 10.0, www.stata.com). A p-value < 0.05 was considered statistically significant. Statistical differences between population prevalence of myopia across a range of age bands (45–49, 50–54, 55–59, 60–64, 65–69, 70–74) at two time intervals were calculated using an ANOVA test in STATA.

Results

The TwinsUK Adult Twin Registry contains 13,511 twin subjects who have volunteered over the last 20 years, and 8960 twin subjects have attended assessments. The registry consists of British Twins who are predominantly female and of Caucasian decent. Autorefraction commenced in 1998, and after 222 exclusions [due to retinal detachment ($n = 28$), cataract surgery ($n = 99$) and/or laser refractive surgery ($n = 114$)] autorefractive data were available on 6097 participants with a mean age of 53 years [standard deviation (S.D.) 12.61, median 55, mode 54, range 16–85]. In the aim of studying and therefore capturing those developing myopia after the age of 17 we have excluded those who were under the age of 20 at their baseline refraction ($n = 48$). The mean S.E. was -0.36 D (S.D. 2.67, median -0.06 D, mode 0 D, range -25.13 to $+9.38$), illustrated in *Figure 1*. Twenty-eight percent were myopic ($n = 1693$) with a mean refractive error of -3.54 D (S.D. 2.51, range -25.13 to -1.00).

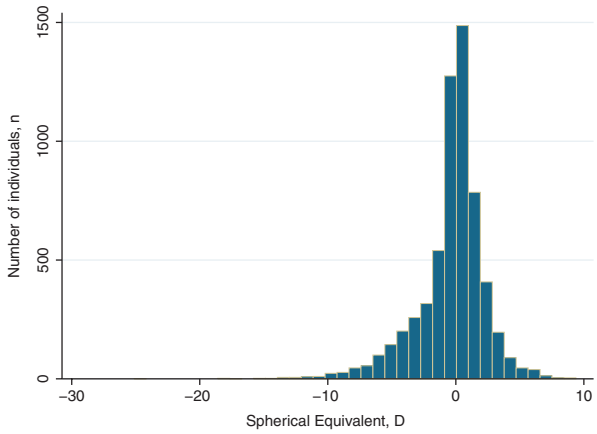

Figure 1. Graph showing distribution of refractive error. D, dioptres.

Questionnaire data regarding age of spectacle wear were available for 4381 of the 6049 subjects with autorefraction following the above exclusions. In those with myopia the age that spectacles were first worn was available for 1465 of the 1693 myopic subjects (87%). The median age of myopia onset was 15.0 years (mean 18.4 years, S.D. 12.24, mode 11, range 0–74) with 42.3% developing myopia at or after the age of 17, as shown in *Table 1*. This study confirms previous work that early onset of myopia is more likely to result in high myopia; over 90% of high myopes in our cohort wore glasses before the age of 17. Only 10% of the cohort developed myopia after the age of 40. This correlation between age of myopia onset and severity is illustrated in *Figure 2*. The correlation coefficient between age of myopia onset and severity was 0.39 ($p < 0.0001$), which equates to an approximate variance of 15% ($r^2 = 0.15$).

Of those who provided an age at which they first wore glasses for myopia in both questionnaires ($n = 628$), there was median difference in response of 0 years (S.D. 7.18, mean 0.7, maximum 53). Answers in response to the first age of spectacles worn in the two questionnaires 5 years apart

Table 1. Distribution of myopia by age at onset and severity

Age at myopia onset (years)	*n* (%)	Mean Spherical Equivalent (D)	Spherical Equivalent S.D.	High myopia *n* (%)	Moderate myopia *n* (%)	Low myopia *n* (%)
<17	846 (57.8)	−4.43	2.78	188 (93.0)	360 (70.1)	298 (39.5)
≥17	619 (42.3)	−2.38	1.37	14 (6.9)	148 (29.1)	457 (60.5)
17 to <20	167 (11.4)	−3.03	1.63	9 (4.6)	63 (12.4)	95 (12.6)
20 to <40	303 (20.7)	−2.32	1.25	5 (2.5)	69 (13.6)	229 (30.3)
40–74	149 (10.2)	−1.78	0.88	0 (0)	16 (3.1)	133 (17.6)
Total	1465	−3.56	2.50	202	508	755

n, number.

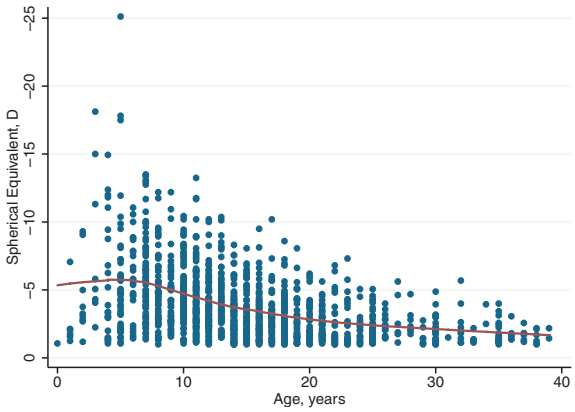

Figure 2. Scatter plot of age at myopia onset (<40 years old) and myopia severity. D, dioptres.

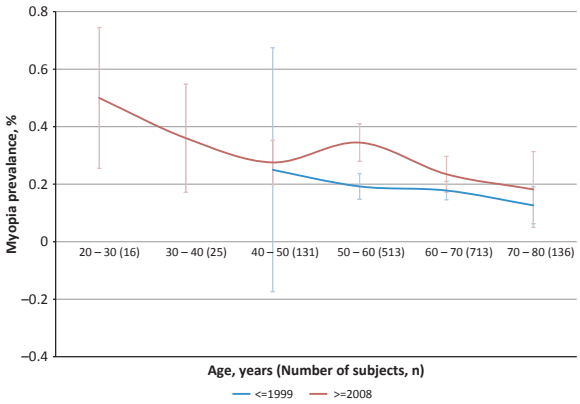

Figure 3. Prevalence of myopia among participants of 1998–1999 examinations and 2008–2010 examinations against age at refraction (95% Confidence Intervals).

was the same in 290 subjects, ≤ 1 year different in 67% of the sample and 96% of responses differed by 5 years or less.

We studied the cohort effect for changes in prevalent myopia between two groups of participant examinations separated by 10 years (1998–1999 and 2008–2010), illustrated in *Figure 3*. In those aged 50–54 years old the prevalence of myopia increased from 27% to 39% and in those aged 55–59 from 16% to 32%. Between the ages of 45 and 70 we found a statistically significant difference in myopia prevalence between 1998–1999 and 2008–2010 across a range of age sub-groups (p-value = 0.02).

Discussion

We have identified a significant proportion of adult onset myopia; 42.3% of those with myopia in our large British twin cohort developed myopia at or over the age of 17. This figure, in our cohort born between 1920 and 1990, is lower than the 1958 British Birth Cohort, where 76% of myopes had good uncorrected vision at the age of 16. However it confirms the finding that a significant number of myopes first get spectacles in adulthood in a United Kingdom cohort. This is in contrast to recent publications on the impact of school onset myopia in younger generations in South East Asia, where the peak age of onset is 12–13.[4,5,13] Recent UK data have found a myopia prevalence of approximately 18% at 12–13 years.[30,31] Only 14.0% (95% CI 12.9–15.0) of our cohort self-reported spectacle wear by that age, also suggesting that age of myopia onset may be getting younger in the UK. Ten percent of the cohort developed myopia after the age of 40. This may be due to changes in axial length in middle aged onset myopia[18] or the increase in myopia observed during the incipient phase of presbyopia in myopes in a recent preliminary study.[32] In those in later life, myopia development is likely to be due to a mixture of cataract induced myopia or the known myopic shift identified to occur in population based studies after the age of 70.[33]

We identified an overall myopia prevalence of 28% utilising autorefractive data collected over a 12-year period in the TwinsUK cohort ranging in age from 16 to 85. Using a matched myopia definition of ≤ -1.00 D, the level of myopia in our cohort was comparable to the crude estimates of 25.4% in the US and 26.6% in Western Europe provided by the Eye Disease Prevalence Research Group.[8] Pooled data from white females in this meta-analysis suggests our cohort, with a mean age of 53, has a slightly higher prevalence in the 50–54 year old age range (30.3% vs 34.0%).

Studies assessing the age range of myopia onset in British adult populations are sparse. In McBrien and Adams[18] paper assessing myopia onset in clinical microscopists, aged 21–63 years old, 33% developed myopia after the age of 20. In a 2005 publication assessing refractive error within a UK university 50% of Caucasian and 53.4% of British Asian students, with a mean age of 19.5, were myopic.[9] Recent international research assessing the proportion of myopia onset in adult populations provides variable findings; 14.7–44.9% in comparative medical student cohorts,[34–36] and 47.8% in office workers.[37] The Genes in Myopia (GEM) study assessed age of myopia onset within Australian twins, aged between 18 and 86.[10] This cohort is comparative to the TwinsUK cohort in that approximately two-thirds were females and the majority were Caucasian. Approximately 30% of their cohort was myopic (n = 347). Adult onset myopia was identified in 27% and all developed low to moderate myopia (range −0.50 to −4.00 D). In our study there were a higher proportion of twins developing adult onset myopia, and we observed 36% to develop 3D or more of myopia in adulthood.

The commercial personal genomics company, 23andMe, recently reported a genome wide association study (GWAS) for myopia, utilising 23 000 cases and 16 000 controls, which replicated two known and identified 17 novel genetic variants.[21] The bias of this cohort should be questioned in that the individuals participating are likely to be more educated, of higher socioeconomic status and more health conscious given that they have self-funded an internet-based genetic study to look at personal health and ancestral prediction. The analysis was undertaken without any refractive data; instead myopia and myopia severity were inferred from self-reported diagnosis and age of glasses requirement respectively. This source of data raises concerns regarding the research validity. However, the astonishing concordance of results with published GWAS[22–24] suggests that the age of glasses wear is a reasonable quantitative proxy of refractive error in a study of this type where large subject numbers are possible but without access to refractive data, although it is likely the data in our cohort are less reliable than the 23andMe cohort.

While our data on age of spectacle wear are retrospective and recall-based, previous research into myopia has suggested the diagnosis of myopia and the age at which glasses were first worn is a highly memorable event; in a Danish study a precise recall of myopia onset was identified in 148 of a sample of 151 adult subjects with detailed accounts to substantiate the event.[38] It would appear that the onset of myopia is a strong emotional experience. This is confirmed in our data where age of myopia onset in over 600 subjects asked 5 years apart was very similar; two-thirds differed within 1 year in their response and though there was some variation (96% of answers were <6 years different), the responses were reasonably reproducible. Although spectacle wear can only be an imperfect proxy for true age of myopia onset, particularly as age of first wear is likely to lag behind age of myopia onset, research has consistently shown that those who require glasses for myopia at a younger age will have a higher degree of final myopia. This was replicated in our data where the age of glasses for myopia was correlated with spherical equivalent, with a correlation coefficient of 0.39 ($p < 0.0001$).

We have found evidence of a statistically significant cohort effect for increasing myopia prevalence in those aged 45–70 years old, inclusive of the majority of subjects in our study. At two time points separated by 10 years (1998–1999 to 2008–2010) in those age 50–55 we observed an increase in myopia prevalence from 27% to 39% and in those aged 55–59 from 16% to 32%. This suggests that there may be more myopia in those born after 1950 in our sample. This might be related to known social factors such as an increase in female education and the increased prosperity and education experience by the 'Baby Boomer' generation after World War 2. Myopia prevalence in the United States was observed to increase over a 30 year period between 1971–1972 and 1999–2004.[3] In those aged 45–54, the eldest age range studied, myopia prevalence increased from 25.5% to 46.2% in Caucasians of mixed gender. This corroborates the trend seen in our data, that there is a cohort effect and myopia is becoming more common. Our data additionally shows myopia prevalence reduces with age; a trend that the Beaver Dam Eye Study concluded was in part due to a cohort effect and longitudinal hyperopic shift.[33]

The subjects in this study are twins, but we do not believe refraction data to be significantly different to singleton data.[39] Twins have also been shown, in general, to have a very similar morbidity and mortality to singletons.[40] The prevalence of myopia in this study was similar to the 1958 British Birth Cohort[1] and the Western European prevalence calculated by the Eye Diseases Prevalence Research Group.[8] Any ascertainment bias was reduced by the fact that the subjects in this study volunteered to be on our research Registry unaware of any specific myopia studies, and phenotyping was performed as part of a larger TwinsUK study, of which autorefraction was a small part. We chose a definition of −1.00 D for myopia to maintain consistency with previous adult studies. Given the age of our cohort, we did not think the lack of cycloplegia a major confounder in refractive data obtained. In fact recent research by Toh *et al.*,[41] suggested an apparent myopic shift is obtained when cycloplegia is used in older subjects whilst an apparent hyperopic shift is found in younger subjects. However we do acknowledge that lack of cycloplegia in autorefraction can lead to measurement errors in young adults; in the Tehran Eye Study it was noted that overestimation of myopia occurred in the 20–40 age group and that more significant underestimation of hyperopia was seen in those up the age of 50.[42] Our cohort had a mean age of 53 and thus the majority were subject to the reduced errors in measurement seen with increasing age. Our cohort has a similar mean age to the Beaver Dam Offspring study, who were noted to have a mean difference in S.E. measurements before and after cycloplegia of 0.29 D (95% CI 0.28–0.31), which reduced with age, and a high agreement on refraction classifications across all ages (84–92%).[43]

This study confirms previous research that myopia affects approximately one-third of the British adult population and that a significant proportion of this develops after the age of 17 (i.e. adult-onset myopia). Consideration should be paid to this to avoid incorrectly classifying individuals as non-myopic, particularly in regard to studies of genetic influence in myopia within younger cohorts. Our findings confirm that those developing myopia at a younger age have a higher final degree of myopia and our analysis of cohort effect over a 10-year period suggests significant increases in myopia prevalence in the UK,

confirming findings in Asia and the United States of America. From a clinical perspective, the ages at which myopia develops – and the population changes in refraction that this and other papers have identified – have important implications in the increasing era of refractive surgery provision. Reported age of glasses is reproducible and reflects severity; however it is a rough proxy that only explains approximately 15% of the variance, and therefore its use is only appropriate in large-scale population studies without access to refraction.

Acknowledgements

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Disclosure

The authors have no competing interests to declare. The data analysis and manuscript preparation was performed by KW. Data collection and analysis was performed by PH, AN, KD & CV. Supervision of the data analysis and editing of the manuscript was undertaken by CH.

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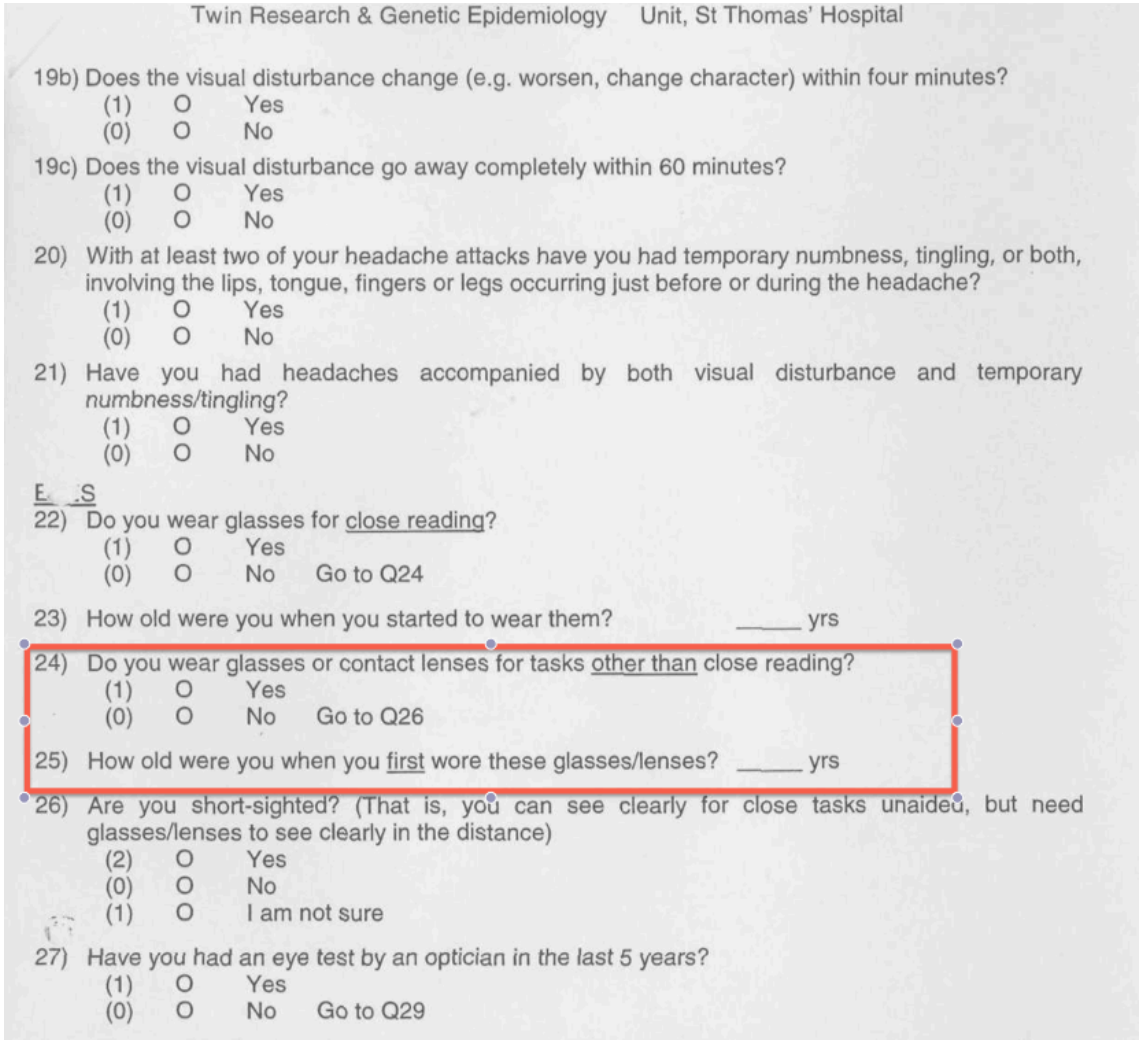
Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. TwinsUK questionnaire conducted between 2002 and 2003.

Appendix S2. 'HATS' questionnaire conducted between 2007 and 2010.

Appendix S1



Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital

19b) Does the visual disturbance change (e.g. worsen, change character) within four minutes?
(1) O Yes
(0) O No

19c) Does the visual disturbance go away completely within 60 minutes?
(1) O Yes
(0) O No

20) With at least two of your headache attacks have you had temporary numbness, tingling, or both, involving the lips, tongue, fingers or legs occurring just before or during the headache?
(1) O Yes
(0) O No

21) Have you had headaches accompanied by both visual disturbance and temporary *numbness/tingling*?
(1) O Yes
(0) O No

E .S

22) Do you wear glasses for close reading?
(1) O Yes
(0) O No Go to Q24

23) How old were you when you started to wear them? _____ yrs

24) Do you wear glasses or contact lenses for tasks other than close reading?
(1) O Yes
(0) O No Go to Q26

25) How old were you when you first wore these glasses/lenses? _____ yrs

26) Are you short-sighted? (That is, you can see clearly for close tasks unaided, but need glasses/lenses to see clearly in the distance)
(2) O Yes
(0) O No
(1) O I am not sure

27) Have you had an eye test by an optician in the last 5 years?
(1) O Yes
(0) O No Go to Q29

Appendix S2

OPHTHALMOLOGY

The branch of medicine concerning the eyes.

1. Have you EVER been told by a doctor or other health professional that you had ANY of the conditions listed below in Questions 2-12?

   YES O (1) **First fill out the Screening Table, *then* continue to Question 13**

   NO O (0) → **Go to Question 13, found below the Screening Table**

SCREENING TABLE (Questions 2-12)

Condition	A	B	C
	Have you ever been told by a doctor or optician that you had…		**Have you ever received treatment or worn glasses for…**
	Tick if yes:	*Age at 1st diagnosis:*	*Tick if yes:*
2. Glaucoma	O	__ __ years	O
3. Cataract	O	__ __ years	O
4. Age-related Macular Degeneration (AMD)	O	__ __ years	O
5. Short-sightedness (myopia)	O	__ __ years	O
6. Squint (one or both eyes turning out)	O	__ __ years	O
7. Lazy (amblyopic) eye (despite correction with glasses, one eye still has worse vision than the other)	O	__ __ years	O
8. Colour blindness	O	__ __ years	O
9. Partial sight	O	__ __ years	O
10. Detached retina	O	__ __ years	O
11. Diabetic retinopathy	O	__ __ years	O
12. Other: ……..	O	__ __ years	O

13. Have you ever had cataract surgery?

Yes, cataract surgery on right eye only	O	(1)
Yes, cataract surgery on left eye only	O	(2)
Yes, cataract surgery on both eyes	O	(3)
No	O	(0)

14. Have you had laser or other eye surgery for short-sight, long-sight, or astigmatism?

Yes, surgery on right eye only	O	(1)
Yes, surgery on left eye only	O	(2)
Yes, surgery on both eyes	O	(3)
No	O	(0)

15. Do you wear eyeglasses/lenses…

Yes, for close reading only?	O	(1)
Yes, to see at a distance only?	O	(2)
Yes, for all purposes (close and distant sight)	O	(3)
No	O	(0) → **Skip to next page**

15. When did you first start wearing eyeglasses or lenses? …….. years old

Chapter 4 | Environmental factors for myopia

4. 1 Introduction

In this Chapter I explore cognitive, behavioural and environmental associations with myopia. The Twins Early Development Study (TEDS) is described more fully in Materials 7.1, where I also describe how I collected the refractive error data, how refractive error was classified, how representative the sample was of the broader TEDS cohort and UK population, and finally how much refractive error was present. In this chapter I present my findings on early life associations with myopia in TEDS using a life course epidemiology approach in a submitted paper. Secondly I report the association between lifetime UVB exposure, vitamin D and myopia in an older adult European study, EUREYE, in a published paper. This research came about following collaborative conversations with other members of the E^3 community and exploitation of existing, large datasets.

4.2 Early life factors for myopia in the Twins Early Development Study

This chapter is presented as a submitted paper and is an exact copy of the following submitted journal article:

Katie M Williams, Eva Krapohl, Ekaterina Yonova-Doing, Pirro Hysi, Robert Plomin, Christopher J Hammond. Early life factors for myopia in the Twins Early Development Study. Under review with *Ophthalmology*

Abstract

Objective: To investigate early life factors associated with developing myopia by the end of childhood

Design: Longitudinal, twin, birth cohort study

Participants: A subset of the UK-based Twins Early Development Study (TEDS) (n=1991) recruited at birth between 1994-1996.

Methods: Extensive phenotype data, with a neurodevelopment focus, has been collected over the twins' early life; candidate myopia risk factors were examined. Subjective refraction data was obtained from the twin's optometrists; mean age 16.3 years (SD 1.7). Heritability was estimated using twin modeling. A life course epidemiology approach was used to appropriately weight exposures during critical periods of early eye growth. Multivariable logistic regression models at each life stage were examined: preconception; perinatal, perinatal and postnatal; pre-school (≤ 4 years); childhood (≤ 11 years); adolescence (≤ 18 years).

Main outcome measure: Adjusted odds ratio (OR) for myopia (≤ -0.75D); total variance explained (r^2) and AUROC statistic of predictive models.

Results: 25.9% (95% CI 24.0-27.8) of participants were myopic, with a mean age at myopia onset of 11.0 years (SD 3.8). Genetic factors explained 86% of trait variance; the remaining 14% was explained by non-shared environmental factors. In the final multivariable model, significant associations with myopia included maternal education at preconception (OR 1.33, 95% CI 1.11-1.59), fertility treatment (OR 0.63, 95% CI 0.43-0.92) and summer birth (OR 1.93, 95% CI 1.28-2.90) from the perinatal period, and hours spent playing computer games (OR 1.03, 95% CI 1.01-1.06) during adolescence. The total variance explained by this model was 6.9% ($p<0.001$) and the AUROC was 0.68 (95% CI 0.64-0.72). Consistent associations were observed with socioeconomic status, educational attainment, reading enjoyment and cognitive variables, particularly verbal cognition, at multiple points over the life course.

Conclusions: This study confirms known and identifies novel associations with myopia during childhood development; some reflect sociological and lifestyle trends such as

rates of maternal education, fertility treatment, early schooling, and computer games. An increased understanding of contemporaneous, early life factors that are associated with myopia risk, particularly earlier onset myopia as this correlates with higher severity and increased ocular complications in adult life is required.

Myopia, or near sightedness, typically occurs when there is axial elongation of the eyeball in childhood resulting in a focused image forming in front of the retinal plane. This requires refractive correction but also continues to place an individual at an increased risk of potentially sight threatening diseases, particularly in the case of high myopia (1). The prevalence of myopia is on the rise worldwide, most dramatically in urban Asian countries (2-4). There is increasing interest in strategies to reduce the development and progression of myopia during childhood (5), which in turn could reduce the potential burden of associated visual impairment in the future.

Before the age of two years there is a period of rapid eye growth (6), correlating with the period during which the typical hyperopia associated with infancy reduces due to emmetropization. Scleral remodeling allows axial growth of the eye to near-adult size by the age of 10 (7). Early neonatal visual experience in animals is highly influential in eye growth and refractive development (8, 9). Non-myopic children with a family history of myopia, and therefore highly likely to become myopic, have longer axial lengths than those without a family history (10). Future myopic status can be predicted by refraction in childhood (11); in a study of children aged 6 to 11 years old the single best predictive factor of future myopic status was spherical equivalent refractive error (12). Even in very young children (6 to 12 months of age) those with refractive errors in the 'lower half' of a study population distribution were 4.3 times more likely to develop myopia compared to those in the upper half (13). Importantly early onset myopia is associated with higher degrees of myopia in adulthood, and therefore a higher risk of associated ocular complications; onset before the age of nine predicts high myopia in adulthood with 73% specificity and 80% sensitivity (14).

Although a child's genetic inheritance is a key determinant of their risk of myopia (15, 16), genetic factors alone cannot explain the rising prevalence of the condition. Given that there is rapid eye growth in early life and that future myopic status is predictably ascertained by increased axial length and refraction in early childhood, this study analyzed various candidate risk factors in childhood development, using a life course epidemiology approach. We aimed to estimate myopia risk using a twin cohort study designed to specifically assess early neurodevelopment, cognition, behavior and education during critical periods of childhood development and growth.

Methods

Study population

The Twins Early Development study (TEDS) is a longitudinal birth cohort of twins studied using multivariate quantitative and molecular genetic methods with a neurodevelopmental focus. In the period 1994 to 1996 over 15,000 families of twins from England and Wales were recruited. Despite some attrition the sample remains representative of the UK population (17). For the TEDS myopia study a subset of 2625 families were selected, inclusive of families where twins had completed a questionnaire containing eyesight questions, and additional families where twins had genotype data. Exclusions included children with severe current medical problems and families who were not contactable or who lived overseas. The Institute of Psychiatry ethics committee (at King's College London) has provided ethical approval for TEDS and TEDS myopia study, and the research adheres to the tenets of the Declaration of Helsinki.

Study variables

Refraction

Postal questionnaires were sent to the subset of families in the TEDS myopia study. Study participation and informed consent to contact the twins' optician for a recent refraction was sought from both the parents and twins. A response rate of 51.7% (n=2715) from potential twin participants was obtained. Non-responders and responders were similar in terms of ethnicity, gender, zygosity, age and parental employment. Among responders there was a higher level of school achievement - 90% of responders achieved higher grades in secondary school examinations compared to 84% in non-responders. 45% of responders (n=1227) reported wearing glasses, of which n=309 reported contact lens wear. Questionnaires were posted to the optometrists of the 2,283 twin participants who had undergone an eye test and provided consent. Optometrists were asked to provide the most recent refraction together with a brief ophthalmic and refractive history. Non-cycloplegic, subjective refractive error measurements were obtained for 1991 individuals. Spherical equivalent (SE) was calculated using the standard formula (SE = sphere + (cylinder/2)) and the mean of the two eyes was considered for each individual. Myopia was defined as SE ≤ -0.75 diopters (D) with low myopia ≤ -0.75 to >-3 D, moderate myopia ≤ -3 to >-6D, and high myopia as ≤ -6 D.

Candidate myopia risk factors over childhood development

The twins and parents have completed extensive questionnaires over the early life course, in addition to teacher questionnaires, web-based testing and home

assessments. We examined candidate myopia risk factors at all available age points, namely at the ages 2, 3, 4, 7, 8, 10, 12, 14 and 16. A priori known and potential candidate myopia risk factors were examined, with some variables examined at multiple life stages. Particular attention was placed on cognitive, behavioral and educational variables. Verbal and non-verbal cognitive ability was examined, together with a measure of general cognitive ability or 'g' (18) which is a composite of at least two tests assessing verbal and non-verbal intelligence, described in more detail previously (19). Additional questionnaire data on extra-curricular interests (hours spent) were examined, with particular attention paid to time spent outside, sports and near work activities such as reading and computer games. Certain variables were derived; notably photoperiod was calculated by downloading "civil twilight" hours (hours of daylight) in London during 1995 from a public repository (http://aa.usno.navy.mil/data/docs/RS_OneYear.php). This enabled construction of four photoperiods of increasing daylight hours at birth, comparable to previous examinations of this variable (20, 21).

Genetic Factors: zygosity and twin modeling of heritability

The parents completed questionnaires of physical similarity; this has shown over 95% accuracy in zygosity assignment when compared to DNA testing (22). If zygosity remained unclear DNA testing was performed. Heritability was calculated by twin modeling with the OpenMx package (http://openmx.psyc.virginia.edu) in R (23, 24). In this technique the variance of a trait is estimated by the contributions of three factors: the additive genetic component (A), the shared environment (C) or the dominant genetic effects (D), and the unique environment (E), the latter also comprising measurement error. Maximum likelihood, structural-equation models were constructed for the quantitative trait of spherical equivalent (adjusted for age and sex). The goodness of fit of the full and reduced models to the observed data was evaluated, and the most parsimonious model is identified.

Statistical analysis

Candidate myopia risk factors were evaluated using a life course approach with five life stages: preconception; prenatal, perinatal and postnatal; pre-school (≤ 4 years); childhood (≤ 11 years); adolescence (≤ 18 years). Univariable and multivariable logistic regression models for the risk of adolescent myopia (≤ -0.75 D vs. > -0.75 D) for predictors at each life stage were constructed, with clustering to adjust for family relatedness. At each life stage the multivariable model incorporated adjustment for age

at refraction, sex and factors found to be significantly associated with myopia at any earlier life stage ($p<0.05$ in the multivariable model). The advantage of this life course epidemiology approach is an appreciation for accumulation of risk over childhood development, to elucidate processes operating across different life stages, and allow appropriate consideration and weighting of the effect of environmental exposures during critical periods of development, and in this setting, ocular growth (25). At the adolescence life stage, myopic status was restricted to those who underwent an eye examination after the age of fourteen to avoid the assessment of candidate risk factors subsequent to the date of measurement of refractive error. The variance explained (r^2) and area under the receiver operator characteristic curve (AUROC) statistic of the final predictive model was observed, with adjustment for multiple testing using Bonferroni correction. Analysis was performed using Stata version 13.1.

Results

Spherical equivalent (SE) was calculated on 1991 twin participants. The median age at refraction was 16.7 years (range 5.7 to 18.8 years); 70% were aged ≥16 years and 92% were aged ≥14 years at their most recent refraction. The mean spherical equivalent was -0.35 D and standard deviation (SD) 1.80, with a range from -10.13 to +10.50 D. The mean age at which glasses or contact lenses were first worn was 10.1 years (SD 4.6), and 11.0 years (SD 3.8) if the twin was myopic. Amblyopia was reported by the examining optician in 5.4% and 4.3% had a documented squint. Overall 25.9% of the TEDS cohort was myopic (95% confidence interval (CI) 24.0 - 27.8). The number of individuals with myopia predictably increased with age [Figure 1] – in those aged 16 to 18 years (n=1393, mean age 17.1 years) 32.4% (95% CI 30.0 - 34.9) had myopia. High myopia was present in 1.2% (95% CI 0.8 - 1.7) overall.

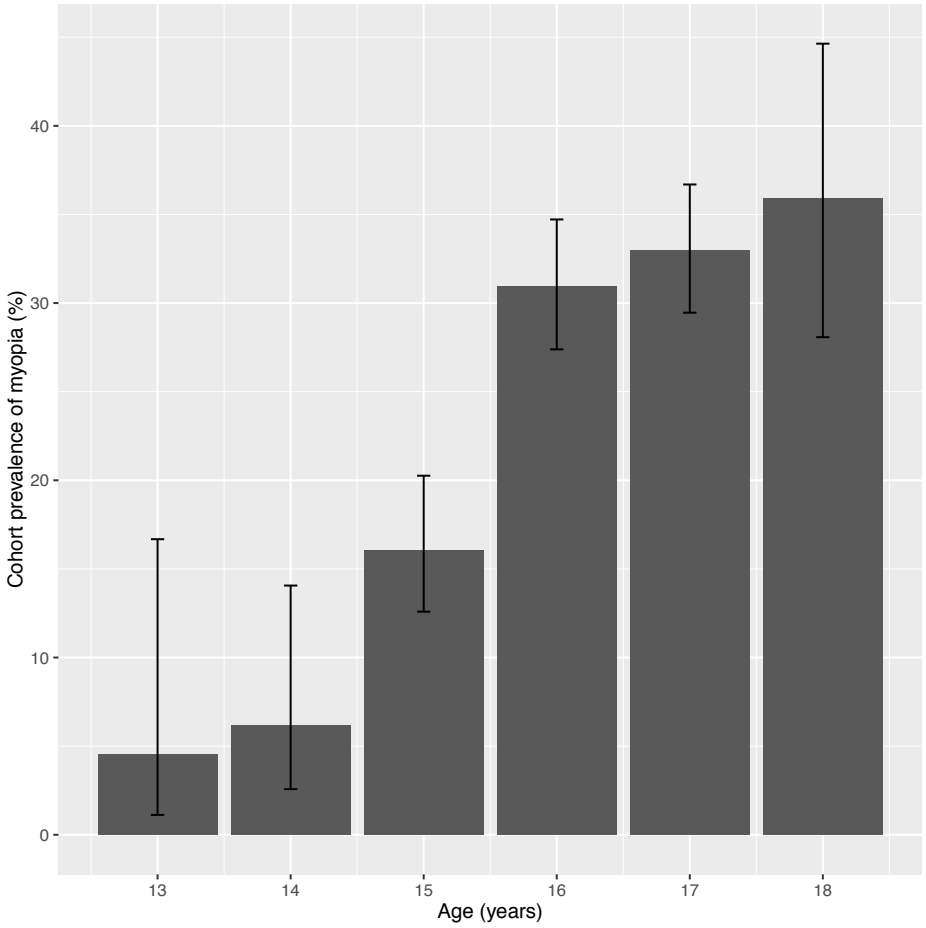


Figure 1 Cohort prevalence of myopia ((≤ -0.75 D) by age (95% confidence intervals)

Genetic Risk

Twin modeling for myopia (adjusted for age and sex) was performed on 333 MZ and 579 DZ twin pairs (within twin pair intraclass correlation coefficients of 0.85 & 0.30 respectively). The best-fit model was 'ADE' from which the heritability estimate, due to a combination of additive and dominant genetic effects, was 85.6% (95% CI 82.6 – 87.7), indicating a high contribution of genetic factors to spherical equivalent variance, whilst environmental effects not shared by twins growing up in the same family were estimated to contribute 14.4% (95% CI 12.3 - 17.0).

Preconception factors

The highest educational level achieved by both the mother and father was significantly associated with myopia in the twins [Table 1]; the odds ratio (OR) for myopia was 1.59 (95% CI 1.00 – 2.51) with a father who attained a university education and 2.15 (95% CI 1.09 – 4.25) with a mother who achieved likewise. In multivariable analyses only the mother's educational attainment remained significant (OR 1.31, 95% CI 1.16 – 1.55). Maternal and paternal educational levels were correlated ($r=0.43$, $p<0.01$), but sensitivity analyses did not affect the results. In univariable analyses there was a significant trend for increased odds of myopia with a father who chose to stay at home to look after children (OR 1.91) or was not working (OR 1.63) compared to an employed father, and also increasing social class, (OR 1.14, 95% CI 1.04 -1.26) as defined by the father's occupation.

Potential risk factor		Myopia (≤ -0.75D)			
	n	Unadjusted model OR (95% CI)[1]	p value / p trend	Adjusted model OR (95% CI)[2] (n=1776)	p value / p trend
Maternal education	1991	1.32 (1.16 - 1.50)	<0.001*	1.31 (1.11 - 1.55)	0.001*
- No qualification	94	1.00		1.00	
- Secondary school exams aged 16 (GCSEs)	913	1.27 (0.65 - 2.49)		1.29 (0.54 - 3.14)	
- Secondary school exams aged 18 (A Levels) /	448	2.14 (1.08 - 4.27)		2.40 (0.97 - 5.95)	
Vocational certificate or diploma	536	2.15 (1.09 - 4.25)	<0.001*	2.08 (0.84 - 5.18)	0.025*
- University degree					
Paternal education	1991	1.15 (1.02 - 1.30)	0.026*	0.99 (0.83 - 1.17)	0.883
- No qualification	169	1.00		1.00	
- Secondary school exams aged 16 (GCSEs)	674	1.22 (0.76 - 1.95)		1.08 (0.62 - 1.90)	
- Secondary school exams aged 18 (A Levels) /	480	1.22 (0.75 - 1.98)		0.87 (0.48 - 1.58)	
Vocational certificate or diploma	668	1.59 (1.00 - 2.51)	0.008*	1.08 (0.58 - 1.98)	0.920
- University degree					
Maternal job	1978				
- Working	959	1.00			
- Staying at home with children	844	0.93 (0.72 - 1.20)			
- Not working	175	0.88 (0.57 - 1.36)	0.411		
Paternal job	1888			collinearity	
- Working	1801	1.00			
- Staying at home with children	28	1.91 (0.70 - 5.23)			
- Not working	59	1.63 (0.88- 3.01)	0.028*		
Higher maternal socioeconomic status based on employment	945	1.13 (0.95 - 1.34)	0.162		
Higher paternal socioeconomic status based on employment	1781	1.14 (1.04 - 1.26)	0.005*	1.06 (0.94 - 1.18)	0.362

Table 1 Preconception factors

[1] Adjusted for family relatedness [2] Adjusted for age at refraction, sex, family relatedness and significant factors in univariable analyses.

Prenatal, perinatal & postnatal factors

Fertility treatment was associated with reduced odds of myopia (0.71, 95% CI 0.54 – 0.94) and this remained significant in the multivariable model [Table 2]. Fertility treatment was moderately correlated with maternal age ($r=0.30$, $p<0.01$), minimally correlated with maternal education ($r=0.05$, $p<0.01$), and inversely correlated with both gestational age (-0.04, $p<0.01$) and birth weight (-0.04, $p<0.01$). When adjusted for all of these correlates, the association between fertility treatment and myopia increased (OR 0.63, 95% CI 0.41 - 0.98). We explored the association between season of birth as defined by academic terms (Autumn - September to December, Spring - January to April, Summer - May to August), and detected a significant increase in risk across successive terms which was retained in multivariable analysis. Those born in the 'summer term' months had the highest odds of future myopia (OR 1.50, 95% 1.11 – 2.05). There was no significant association between month or photoperiod at birth (i.e. an increasing number of daylight hours at birth), nor mediation of the association between season of birth and myopia by birth weight. Season of birth remained a significant factor in multivariable analysis. Those not of white British ethnic group had nearly double the odds of myopia (OR 1.85, 95% 1.11 – 3.09) in univariable analysis; sub-classification of ethnicity was not possible, although numbers of non-white ethnicity were small (n=85).

Potential risk factor		Myopia (≤ -0.75D)			
		Unadjusted model		Adjusted model	
	n	OR (95% CI)[1]	p value / p trend	OR (95% CI)[2] (n=1977)	p value / p trend
Smoking during pregnancy (cigarettes/day)	1965	0.87 (0.66 – 1.17)	0.380		
Alcohol during pregnancy (units/week)	1931	0.98 (0.81 – 1.18)	0.834		
Age of mother	1964	1.01 (0.98 – 1.03)	0.610		
Fertility treatment	1982				
- No	1442	1.00		1.00	
- Yes	540	0.71 (0.54 – 0.94)*	0.017*	0.75 (0.57 – 1.00)	0.047*
Gestational age at birth	1952	0.98 (0.93 – 1.02)	0.349		
Ethnic group of twin	1989				
- White British	1904	1.00		1.00	
- Other	85	1.85 (1.11 – 3.09)*	0.018*	1.52 (0.90 – 2.58)	0.120
Gender	1991				
- Female	1156	1.00			
- Male	835	0.88 (0.70 -1.11)	0.273		
Birth-weight (adjusted for gestational age and gender)	1953	1.00 (1.00 -1.00)	0.962		
Length at birth (adjusted for gestational age and gender)	989	1.01 (0.97 – 1.06)	0.593		
Month of birth	1991	0.99 (0.96 – 1.03)	0.644		
Photoperiod of birth (increasing daylight hours)	1991	1.07 (0.96 – 1.19)	0.237		
- 1	611	1.00			
- 2	475	0.91 (0.65 – 1.27)			
- 3	450	1.03 (0.74 – 1.44)			
- 4	455	1.21 (0.87 – 1.68)	0.149		
Season (by Academic term) of birth	1991	1.18 (1.02 – 1.37)	0.024*	1.22 (1.05 – 1.43)	0.011*
- Autumn	772	1.00		1.00	
- Spring	625	1.19 (0.89 – 1.58)		1.08 (0.80 – 1.46)	
- Summer	594	1.40 (1.05 – 1.89)	0.006*	1.50 (1.11 – 2.05)	0.007*
Breastfed (y/n)	1944	1.04 (0.80 – 1.35)	0.784		
Regular sleeping pattern (y/n)	1945	0.95 (0.82 – 1.10)	0.478		
Length of stay in special care baby unit after birth (days)	736	1.00 (0.98 – 1.01)	0.572		
Length of stay in hospital after birth (days)	1932	1.00 (0.99 – 1.01)	0.993		

Table 2 Prenatal, perinatal and postnatal factors
[1] Adjusted for family relatedness [2] Adjusted for age at refraction, sex, family relatedness, significant factors in univariable analyses & factors significant in adjusted analyses at any earlier life stages. Significance thresholds: * = p-value <0.05, † = p-value <0.10

Pre-school factors

A large number of potential risk factors at this life stage were explored given that this is a critical period for eye growth; the human eye reaches 90% of adult size by four years of age. These included tests of vocabulary, reading ability, language, concentration, attention, reading, outdoor play, anthropometry and cognition. However, only problems with eyesight at the age of three were associated with myopia in univariable analyses and remained significant (p=0.002) in multivariable models [Supplementary Table 1]. Reported problems with eyesight at the age of three (n=56) were associated with a large reduction in odds of myopia (OR 0.23, 95% CI 0.09 - 0.60 in the multivariable model). This is likely due to the identification of children with significant hyperopia, who are unlikely to emmetropize or become myopic; the mean refractive error of these individuals in adolescence was +1.96 D.

Potential risk factor		Myopia (≤ -0.75D)			
		Unadjusted model		Adjusted model	
	n	OR (95% CI)[1]	p value / p trend	OR (95% CI)[2] (n=1072)	p value / p trend
Age 2 Cognitive ability (g)	1107	0.95 (0.80 – 1.12)	0.525		
Age 2 Vocabulary total score	1197	1.00 (0.99 – 1.00)	0.172		
Age 2 Word use total score	1184	1.00 (0.87 – 1.14)	0.964		
Age 2 Sentence complexity score	1184	1.00 90.94 – 1.05)	0.940		
Age 2 Anxiety	1192	0.94 (0.87 – 1.01)	0.085†		
Age 2 Hyperactivity problems	1191	0.96 (0.89 – 1.04)	0.327		
Age 2 Restless	1190	0.84 (0.68 – 1.04)	0.105		
Age 2 Poor concentration	1185	0.90 (0.72 – 1.11)	0.312		
Age 2 Inattentive	1148	1.07 (0.81 – 1.40)	0.645		
Age 3 Cognitive ability (g)	1147	0.98 (0.83 – 1.15)	0.803		
Age 3 Number of children's books	1328	0.99 (0.85 – 1.15)	0.877		
Age 3 Use of outdoor toys (always, usually, no)	1340	0.98 (0.74 – 1.30)	0.903		
Age 3 Problems with eyesight					
- No	1261	1.00		1.00	
- Yes	56	0.25 (0.10 – 0.65)	0.004*	0.23 (0.09 - 0.60)	0.002*
Age 3 Vocabulary total score	1235	0.99 (0.99 -1.00)	0.071†		
Age 3 Word use total score	1229	0.97 (0.92 – 1.03)	0.352		
Age 3 Sentence complexity score	1228	0.99 (0.95 – 1.03)	0.583		
Age 3 Verbal cognition	1225	0.95 (0.82 – 1.11)	0.543		
Age 3 Non-verbal cognition	1300	1.08 (0.93 – 1.26)	0.310		
Age 3 Anxiety	1330	0.95 (0.90 – 1.02)	0.150		
Age 3 Hyperactivity	1331	1.03 (0.96 – 1.10)	0.463		
Age 3 Restless	1327	1.10 (0.91 – 1.33)	0.321		
Age 3 Poor concentration	1329	1.04 (0.85 – 1.28)	0.696		
Age 3 Inattentive	1316	1.01 (0.80 – 1.28)	0.910		
Age 3 Height	1144	1.67 (0.10 - 28.34)	0.722		
Age 3 Weight	1169	0.94 (0.88 - 1.01)	0.104		
Age 4 Cognitive ability (g)	1595	1.01 (0.87 -1.17)	0.869		
Age 4 Verbal cognition	1678	1.00 (0.86 – 1.15)	0.997		
Age 4 Non-verbal cognition	1700	1.07 (0.93 – 1.22)	0.329		
Age 4 Problems with eyesight	1743	0.68 (0.41 – 1.12)	0.130		
Age 4 Number of children's books	1725	0.96 (0.84 – 1.11)	0.589		
Age 4 Physical games	1722	0.98 (0.87 – 1.10)	0.740		
Age 4 Use of outdoor toys (always, usually, no)	1729	1.25 (0.98 – 1.62)	0.070†		
Age 4 Likes being read to (y/n)	1710	1.95 (0.25 – 15.40)	0.527		
Age 4 Ability to sound out letters	1686	0.96 (0.82 – 1.11)	0.565		

Age 4 Ability to sound out words	1689	1.00 (0.75 – 1.33)	0.988
Age 4 Ability to tell what a word means	1692	1.31 (0.91 – 1.88)	0.149
Age 4 Restless	1712	1.00 (0.85 – 1.17)	0.954
Age 4 Easily distracted	1708	0.99 (0.83 – 1.18)	0.925
Age 4 Sees task through	1708	0.94 (0.79 – 1.12)	0.477
Age 4 Inattentive	1689	1.06 (0.85 – 1.32)	0.599
Age 4 Picture vocabulary total score	1708	0.99 (0.90 – 1.09)	0.852
Age 4 Vocabulary total score	1719	1.00 (0.98 – 1.02)	0.784
Age 4 Anxiety	1710	0.99 (0.92 – 1.06)	0.744
Age 4 Hyperactivity	1713	1.02 (0.96 – 1.09)	0.514
Age 4 Height	1451	0.28 (0.02 - 3.27)	0.312
Age 4 Weight	1481	0.97 (0.92 - 1.02)	0.242

Supplementary Table 1 Preschool factors

[1] Adjusted for family relatedness [2] Adjusted for age at refraction, sex, family relatedness, significant factors in univariable analyses & factors significant in adjusted analyses at any earlier life stages. Significance thresholds: * = p-value <0.05, † = p-value <0.10. Abbreviations: OR = odds ratio, CI = confidence interval, g = composite score of cognitive ability.

Childhood factors

Significant associations for increased odds of myopia at the age of seven were identified for current maternal qualifications (OR 1.10, 95% CI 1.02 - 1.17) and a non-working father (full time parent OR 2.01, non-working father OR 1.97, p-trend = 0.002) [Supplementary Table 2]. Neither was significant in the multivariable model, not surprisingly as the multivariable model includes and retains significant factors from earlier life stages including preconception maternal education. Verbal cognitive ability at the age of ten was associated with myopia (OR 1.29, 95% 1.08 -1.55), as was the composite score of cognition (g) (OR 1.22, 95% CI 1.01 -1.47). Non-verbal fluency was not a significant predictor, suggesting that the association with the composite score of cognition was largely driven by the verbal cognition. Given that 'g' is a composite of verbal and nonverbal cognition we omitted this from the multivariable model, where verbal cognition was nominally associated (OR 1.23, 95% CI 0.97 - 1.56, p=0.081).

Potential risk factor	Myopia (≤ -0.75D)				
		Unadjusted model		Adjusted model	
	n	OR (95% CI)[1]	p value / p trend	OR (95% CI)[2] (n=881)	p value / p trend
Age 7 Mother highest qualification level	1707	1.10 (1.02 - 1.17)	0.009*	0.91 (0.76 - 1.08)	0.259
Age 7 Father highest qualification level	1511	1.06 (0.99 - 1.14)	0.079†		
Age 7 Working mother	1788				
- Working	1278	1.00			
- Full time parent	434	0.71 (0.53 - 0.97)			
- Not working	76	1.04 (0.58 - 1.88)	0.099†		
Age 7 Working father	1659				
- Working	1555	1.00		1.00	
- Full time parent	83	2.01 (1.15 - 3.51)		1.39 (0.69 - 2.79)	
- Not working	21	1.97 (0.62 - 3.51)	0.002*	1.31 (0.27 - 6.34)	0.337
Age 7 Cognitive ability (g)	1371	1.13 (0.97 - 1.30)	0.108		
Age 7 Verbal cognitive ability	1372	1.12 (0.97 - 1.29)	0.113		
Age 7 Non-verbal cognitive ability	1382	1.09 (0.94 - 1.26)	0.250		
Age 7 Easily distracted (parent questionnaire)	1794	0.99 (0.85 - 1.16)	0.928		
Age 7 Easily distracted (teacher questionnaire)	1593	1.00 (0.83 - 1.19)	0.961		
Age 7 Restless & overactive (teacher questionnaire)	1593	1.08 (0.88 - 1.33)	0.464		
Age 8 Conners inattention scale	1744	1.01 (0.99 - 1.04)	0.264		
Age 10 Cognitive ability (g)	1079	1.22 (1.01 - 1.47)	0.038*	collinearity	
Age 10 Verbal cognitive ability	1079	1.29 (1.08 - 1.55)	0.005*	1.23 (0.97 - 1.56)	0.081†
Age 10 Non-verbal cognitive ability	1080	1.09 (0.91 - 1.31)	0.366		

Supplementary Table 2 Childhood factors

[1] Adjusted for family relatedness [2] Adjusted for age at refraction, sex, family relatedness, significant factors in univariable analyses & factors significant in adjusted analyses at any earlier life stages. Significance thresholds: * = p-value <0.05, † = p-value <0.10. Abbreviations: OR = odds ratio, CI = confidence interval, g = composite score of cognitive ability.

Adolescence factors

Myopia was again associated with verbal cognition at age twelve (OR 1.22, 95% CI 1.06 - 1.40) and age fourteen (OR 1.04, 95% CI 1.01 - 1.07). At the age of sixteen myopia was associated with the composite cognitive ability 'g' (OR 1.30, 95% CI 1.12 - 1.49), verbal cognition (OR 1.06, 95% CI 1.03 -1.10), and non-verbal cognition (OR 1.04, 95% CI 1.01 - 1.08). No cognitive variable at this life stage was significant in the multivariable model, including when correlated cognitive variables were excluded. Hours spent on computer games at the age of fourteen were associated with myopia in both univariable (OR 1.02, 95% CI 1.00 -1.04) and multivariable analyses (OR 1.06, 95% CI 1.02 - 1.10). Hours spent reading at the same age showed a trend towards increased odds of myopia although this was not significant; however, the rating of reading enjoyment was significant in univariable analysis (OR 1.14, 95% CI 1.04 -1.26). No association between physical sports, watching TV or playing outside with friends was observed. Examinations across a number of subjects undertaken at the age of sixteen by all children completing secondary school education, known in the UK as 'General Certificates of Secondary Education (GCSEs)' scores were associated with myopia; both the number of higher grades achieved (OR 1.05, 95% CI 1.00 - 1.10) and the total point score (OR 1.01, 95% CI 1.00 - 1.01). The more significant measure of GSCSE performance (total point score) was carried forward into the multivariable model and was not significant. Height at age sixteen was associated with reduced myopia odds (OR 0.98, 95% CI 0.96 - 0.99), contrary to the expected association direction (26, 27), and not retained in the multivariable model.

Potential risk factor	Myopia (≤ -0.75D)				
		Unadjusted model		Adjusted model	
	n	OR (95% CI)[1]	P value / p trend	OR (95% CI)[2] (n=449)	p value / p trend
Age 12 Cognitive ability (g)	1489	1.11 (0.96 - 1.27)	0.148		
Age 12 Verbal cognitive ability	1512	1.22 (1.06 - 1.40)	0.005*	0.72 (0.48 - 1.08)	0.112
Age 12 Non-verbal cognitive ability	1489	0.98 (0.86 - 1.12)	0.756		
Age 12 Conners inattention scale	1259	1.01 (0.98 - 1.03)	0.664		
Age 14 Physical sports (hours/week)	1147	0.98 (0.95 - 1.01)	0.175		
Age 14 Computer games (hours/week)	1086	1.02 (1.00 - 1.04)	0.015*	1.06 (1.02 - 1.10)	0.003*
Age 14 Watching TV (hours/week)	1126	1.00 (0.99 - 1.02)	0.749		
Age 14 Outside with friends (hours/week)	1049	1.00 (0.97 - 1.02)	0.824		
Age 14 Reading (hours/week)	1060	1.01 (0.99 -1.04)	0.206		
Age 14 Reading (increasing rating of enjoyment 1 - 6)	1206	1.14 (1.04 - 1.26)	0.006*	1.21 (0.97 - 1.51)	0.091†
Age 14 English teacher assessment	1039	1.08 (0.92 - 1.26)	0.342		
Age 14 Maths teacher assessment	1047	1.10 (0.95 - 1.26)	0.196		
Age 14 Science teacher assessment NC	1040	1.07 (0.91 - 1.25)	0.422		
Age 14 Cognitive ability (g)	882	1.16 (0.97 - 1.38)	0.097†		
Age 14 Ravens web test (non-verbal)	895	1.02 (0.97 - 1.06)	0.448		
Age 14 Vocabulary web test	1055	1.04 (1.01 - 1.07)	0.023*	1.02 (0.95 - 1.08)	0.619
Age 16 Household income category (based on both parents)	1040	1.04 (0.97 - 1.10)	0.269		
Age 16 Father highest qualification level	1133	1.02 (0.99 - 1.04)	0.273		
Age 16 Father socioeconomic level	1011	1.06 (0.99 - 1.14)	0.090†		
Age 16 Mother highest qualification level	1220	1.10(1.02 - 1.18)	0.012*	0.80 (0.63 - 1.01)	0.058†
Age 16 Mother socioeconomic level	1050	1.07 (0.99 - 1.16)	0.071†		
Age 16 No. of GCSEs passes at grades A* to C	1747	1.05 (1.00 -1.10)	0.045*	collinearity	
Age 16 Total point score for GCSEs	1747	1.01 (1.00 - 1.01)	0.002*	1.01 (0.99 - 1.02)	0.427
Age 16 Cognitive ability (g)	1067	1.30 (1.12 - 1.49)	<0.001*	1.23 (0.92 - 1.64)	0.155
Age 16 Ravens web test (non-verbal)	1094	1.04 (1.01 - 1.08)	0.018*	collinearity	
Age 16 Mill Hill vocabulary web test	1155	1.06 (1.03 -1.10)	<0.001*	collinearity	
Age 16 Height	980	0.98 (0.96 - 0.99)	0.010*	0.97 (0.94 - 1.01)	0.176
Age 16 Weight	980	0.99 (0.98 - 1.01)	0.275		

Table 3 Adolescence factors [1] Adjusted for family relatedness [2] Adjusted for age at refraction, sex, family relatedness, significant factors in univariable analyses & factors significant in adjusted analyses at any earlier life stages. Significance thresholds: * = p-value <0.05, † = p-value <0.10. Abbreviations: OR = odds ratio, CI = confidence interval, GCSEs = General Certificate of Secondary Education (secondary school exams taken at the age of 16 in the UK).

Significant factors in multivariable analysis at each life stage were combined into one single model in 1077 individuals, with adjustment for age and sex [Figure 2]. The following factors remained significant: maternal education (OR 1.33, 95% CI 1.11-1.59) at preconception, fertility treatment (OR 0.63, 95% CI 0.43 - 0.92) and summer birth (1.93, 95% CI 1.28 - 2.90) from the perinatal period, and hours spent playing computer games (OR 1.03, 95% CI 1.01-1.06) during adolescence. An older age at refraction (OR 1.75, 95% CI 1.47 -2.10) and being female (OR for males 0.70, 95% CI 0.50 – 0.98) were also associated with myopia. The total variance explained by this model was 6.9% ($p<0.001$) and the AUROC was 0.68 (95% CI 0.64 - 0.72), illustrated in Figure 3.

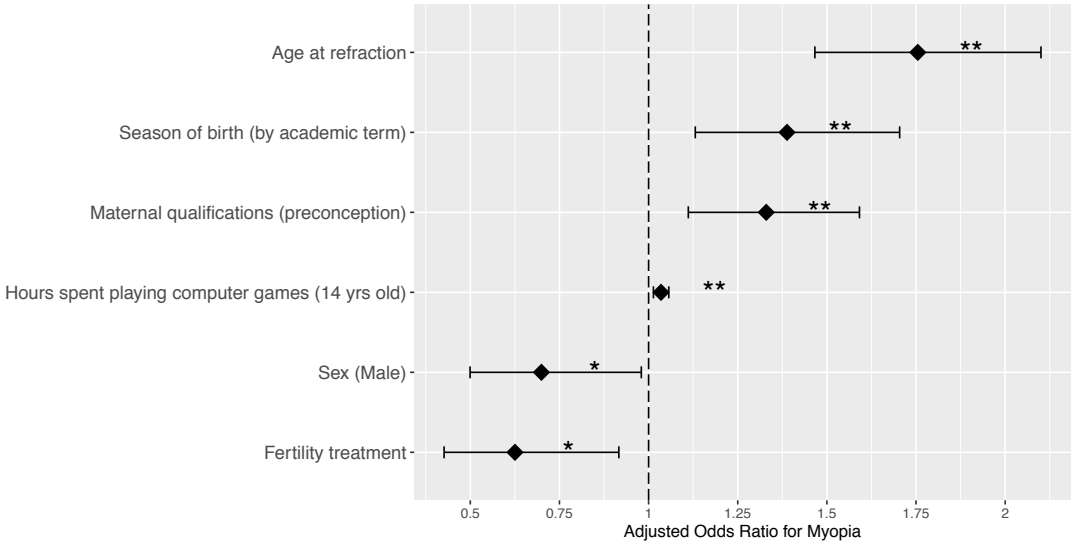


Figure 2 Predictors for myopia from the overall life course analysis (adjusted odds ratio for myopia with 95% confidence interval). Significant factors = *; significant factors after Bonferroni correction = **

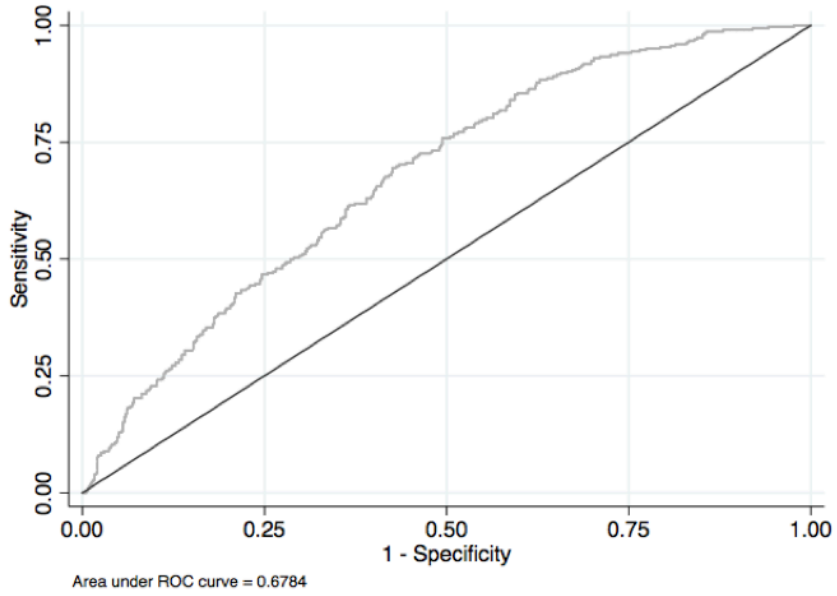


Figure 3 Receiver operating characteristic curves for prediction of myopia

Discussion

In this paper we have attempted to address the question of what early life factors in modern day childhood contribute to myopia. Key associations with myopia as children enter adulthood were maternal education, and a summer birth, whilst reduced risk was observed with fertility treatment. Suggestive associations across the whole of childhood were found with higher socioeconomic status and cognitive scores (akin to intelligence), in particular verbal cognition at younger ages. Measures related to near-work or outdoor activities were not robustly associated with myopia in this study, similar to the findings of others (28), however the quality of these variables was poor. We found hours spent playing computer games were a significant risk factor. Trajectories for ocular growth appear to be influenced by key factors in childhood; this echoes the findings of a previous life course study (26) but with novel findings.

We found, like other studies, a consistent association between the highest educational qualification achieved by the mother and risk of myopia in her child (28, 29). This probably reflects several (mutually inclusive) influences including parenting style, socioeconomic status, wealth, educational encouragement, and potentially shared genetic factors. Notably this association was replicated at multiple stages using a life course epidemiology approach, rather than simply using the variable as a covariate,

highlighting the importance of maternal education at preconception on the future trajectory of her unborn child's ocular growth.

Maternal fertility treatment was inversely associated with myopia, a novel finding. Twin participants are conceived in higher numbers in fertility-assisted conceptions (30). Somewhat contrary to the expectation that women undergoing fertility treatment have more risk factors for myopia (being older due to the pursuit of higher qualifications, or able to afford treatment due to a higher socioeconomic status), we observed a 25-30% reduction in odds of myopia with fertility treatment, which remained despite adjustment for possible confounders. This reduction could, in part, be related to the fact that infants born to mothers who have undergone fertility treatment tend to have a lower birth weight and shorter gestation (31) and have, in some but not all studies, developmental delay and reduced cognitive scores (32, 33). This novel finding requires replication.

In the UK children start school in the September of the academic year in which they turn five years old. Therefore, those born in the summer could be almost a whole calendar year younger than those born in autumn. In this study children entering the educational system at a younger age (born in the summer months) had the highest odds of myopia. Previous studies of Finnish, Israeli, British, and American populations also identified increased myopia with summer births, with several studies attributing this to increased exposure to natural light during the early postnatal period (20, 21, 34, 35). We find no association with increasing light levels at birth and propose that the association between season of birth may be attributable to early exposure of schooling and the educational system. Season of birth has been shown to have long-lasting associations with educational outcomes (36, 37). It may also be that educated parents plan conception because of this, or to plan parental leave to coincide with better weather or less demanding workloads.

Hours spent playing computer games in early adolescence increased the odds of being myopic. When the twins were answering this question (in 2008), which predates hand-held tablets (except the *Nintendo DS*), most computer games consoles were played indoors on television screens avoiding direct sunlight (such as the *NintendoWii*®, *PlayStation2*® and *X-Box*®). This association has previously been reported when included in a total sum of 'near-work hours' (38), but not consistently when considered separately (39). In one study, time spent computer gaming did not vary between future

myopes and emmetropes when measured before myopia onset (40). We did not replicate the protective effects of time outdoors (41, 42); this association was only reported in 2008 and was not carefully measured in this cohort. We found an association with reading enjoyment in univariable analyses. The 'liking' of reading has previously shown to be correlated with myopia (43); we suggest this trait may not simply reflect time spent on the near-work activity of reading, but something in the broader behavior or personality of those children, as others suggested (44). We suppose that the computer games association may similarly reflect personality and behavior traits and a correlation with less time spent outdoors. Further analyses could include estimation of the degree of genetic mediation between playing computer games and myopia using the twin model design.

Over the life course consistent associations between myopia and verbal cognition were observed, also overall cognitive ability (akin to intelligence) but this includes verbal cognition. Generally these associations were not statistically significant at early ages, possibly reflecting the difficulty in measuring these parameters in young children, and therefore were not retained in multivariable models, but there is a clear trend in association [Figure 4]. Intelligence and educational achievement are established risk factors for myopia (43, 45-48). This study highlights that verbal cognitive ability appears to be more strongly associated with myopia than non-verbal, something not previously examined.

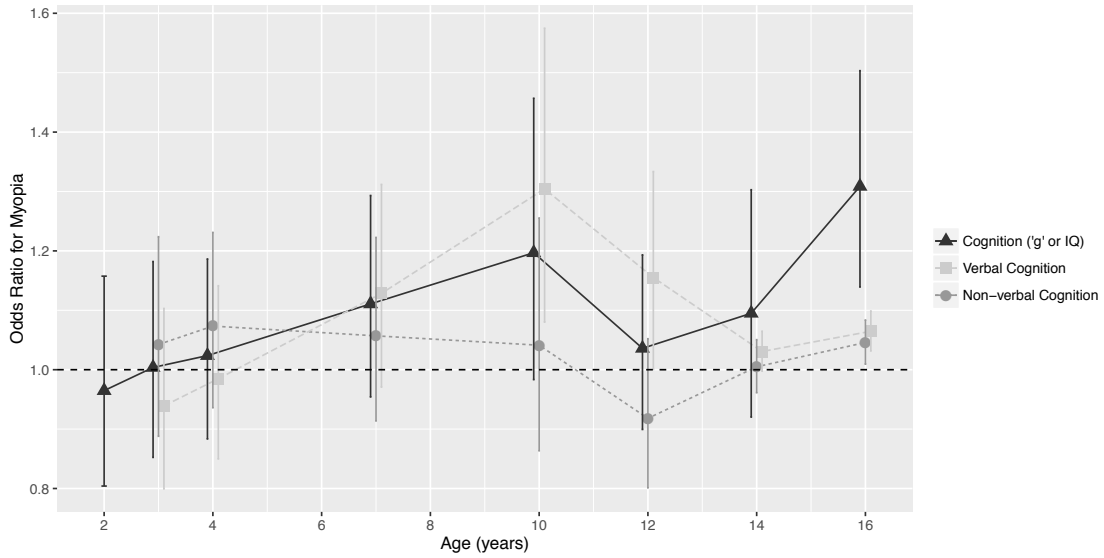


Figure 4 Association between myopia and cognitive ability over childhood; odds ratios for myopia adjusted for age, sex and family structure. General cognitive ability ('g', akin to intelligence quotient 'IQ') is a composite score of tests of verbal and non-verbal intelligence

The age of myopia onset (11 years), as defined by the start of glasses wear, was comparable to similar cohorts (11, 49), and notably younger than historical studies in the UK (26, 50). Twin modeling estimated 14% of trait variation was explained by non-shared environmental factors. Detailed risk factor models assessed in this study explained ~7% of variance, although it should be noted that many of the risk factors in this model may have a genetic component, for example maternal education. This is comparable to previous study estimates of 2-12%, generally comprised of near work variables and notably often including parental myopia (which includes genetic risk) (39, 45). Predictive models have been tested in longitudinal studies (12, 51, 52); AUC statistics achieved range from 0.82 - 0.93 using model parameters including ocular biometry, visual acuity, parental myopia and visual activity. The AUC statistic in our study of life-course risk factors was 0.68, without being able to include data on ocular biometry and parental myopia.

This study comprises a large, contemporaneous, longitudinal study of UK-representative adolescents. Findings may be population-specific and as such associations may be not be generalizable to other samples. Limitations include reduced power due to missing data; numerous assessments of environmental, biological and

social determinants of myopia risk were explored over the life course, in the setting of a wider study on neurodevelopment, and refractive error data was only available for a subset of the whole cohort. The myopia study was not initiated at the start of the TEDS study, and as such questionnaires were not designed specifically to target potential risk factors for myopia. As the oldest participants refracted were 18 years (who could still become myopic), misclassification of adult myopic status may have occurred; importantly, however, this methodology is likely to have captured all of the more highly myopic individuals, who are of most clinical interest. The myopia prevalence according to age may be affected by drop-out of non-myopic individuals at older ages - ie. emmetropic participants may be less likely to attend an optometrist for follow-up refractions as they get older. Therefore the true prevalence of myopia in this cohort may be lower than the estimated 25.9%. Subjective, non-cycloplegic refractions by experienced practicing optometrists were used, hopefully reducing error (compared to non-cycloplegic autorefraction which may overestimate myopia prevalence). Finally, these analyses identify associations with myopia, but do not imply a causal direction. Importantly, the correlations between various early life factors and myopia could be mediated by a latent factor, such as genetics.

In conclusion this study of a contemporaneous, longitudinal adolescent cohort confirms the high heritability of myopia and highlights maternal education, a summer birth, and hours spent playing computer games as key predictors of myopia as child enters adulthood. A negative association with myopia was observed for the prior use of fertility treatment. Consistent associations were observed with socioeconomic factors, educational attainment, reading enjoyment and cognitive variables, particularly verbal cognition, at multiple points over the life course. The majority of trait variance is explained by genetic factors, however changing trends in population prevalence are likely due to changing environmental pressures. Subsequent studies of this and other cohorts are warranted, in conjunction with genetic data, to continue efforts to identify predictive models that can ascertain who should be targeted for treatment (such as increased time outdoors which predominantly reduces the development of myopia rather than progression rates) to reduce the future burden of this condition.

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4.3 The association between myopia, ultraviolet B exposure, and vitamin D

This chapter is presented as a published paper and is an exact copy of the following journal publication:

Katie M Williams, Graham C.G. Bentham, Ian S. Young, Ann McGinty, Gareth McKay, Ruth Hogg, Christopher J Hammond, Usha Chakravarthy, Mati Rahu, Johan Seland, Gisele Soubrane, Laura Tomazzoli, Fotis Topouzis, Astrid E. Fletcher. The association between myopia, ultraviolet B exposure, serum vitamin D and genetic polymorphisms in vitamin D metabolic pathways in a multicountry European study. JAMA Ophthalmol. 2017 Jan 1;135(1):47-5

Research

JAMA Ophthalmology | Original Investigation

Association Between Myopia, Ultraviolet B Radiation Exposure, Serum Vitamin D Concentrations, and Genetic Polymorphisms in Vitamin D Metabolic Pathways in a Multicountry European Study

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IMPORTANCE Myopia is becoming increasingly common globally and is associated with potentially sight-threatening complications. Spending time outdoors is protective, but the mechanism underlying this association is poorly understood.

OBJECTIVE To examine the association of myopia with ultraviolet B radiation (UVB; directly associated with time outdoors and sunlight exposure), serum vitamin D concentrations, and vitamin D pathway genetic variants, adjusting for years in education.

DESIGN, SETTING, AND PARTICIPANTS A cross-sectional, population-based random sample of participants 65 years and older was chosen from 6 study centers from the European Eye Study between November 6, 2000, to November 15, 2002. Of 4187 participants, 4166 attended an eye examination including refraction, gave a blood sample, and were interviewed by trained fieldworkers using a structured questionnaire. Myopia was defined as a mean spherical equivalent of −0.75 diopters or less. Exclusion criteria included aphakia, pseudophakia, late age-related macular degeneration, and vision impairment due to cataract, resulting in 371 participants with myopia and 2797 without.

EXPOSURES Exposure to UVB estimated by combining meteorological and questionnaire data at different ages, single-nucleotide polymorphisms in vitamin D metabolic pathway genes, serum vitamin D_3 concentrations, and years of education.

MAIN OUTCOMES AND MEASURES Odds ratios (ORs) of UVB, serum vitamin D_3 concentrations, vitamin D single-nucleotide polymorphisms, and myopia estimated from logistic regression.

RESULT Of the included 3168 participants, the mean (SD) age was 72.4 (5) years, and 1456 (46.0%) were male. An SD increase in UVB exposure at age 14 to 19 years (OR, 0.81; 95% CI, 0.71-0.92) and 20 to 39 years (OR, 0.7; 95% CI, 0.62-0.93) was associated with a reduced adjusted OR of myopia; those in the highest tertile of years of education had twice the OR of myopia (OR, 2.08; 95% CI, 1.41-3.06). No independent associations between myopia and serum vitamin D_3 concentrations nor variants in genes associated with vitamin D metabolism were found. An unexpected finding was that the highest quintile of plasma lutein concentrations was associated with a reduced OR of myopia (OR, 0.57; 95% CI, 0.46-0.72).

CONCLUSIONS AND RELEVANCE Increased UVB exposure was associated with reduced myopia, particularly in adolescence and young adulthood. The association was not altered by adjusting for education. We found no convincing evidence for a direct role of vitamin D in myopia risk. The relationship between high plasma lutein concentrations and a lower risk of myopia requires replication.

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Supplemental content

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Myopia, or short-sightedness, is a complex trait influenced by numerous environmental and genetic factors. Myopia is becoming more common worldwide, most dramatically in urban Asia, but rises in prevalence have also been identified in the United States and Europe.[1,2] This has major implications, both visually and financially, for the global burden from this potentially sight-threatening condition.

An increased risk of myopia has been associated with urbanization, higher socioeconomic status, prenatal factors, near work, and education.[2-5] The protective effect of time outdoors on myopia has been identified in studies of school-aged children and young adults, with replication in different climates.[6-10] A meta-analysis of 7 cross-sectional studies[11] concluded that there was a 2% reduced odds of myopia per additional hour of time spent outdoors per week. The recommendation for children to spend time outdoors provides an attractive option, and intervention studies are in progress.[12] However, it remains unclear which of the numerous elements associated with time spent outdoors, such as light intensity, ultraviolet radiation (UVR), or distant focus, confers the reduced risk of myopia. Vitamin D concentrations have been inversely associated with myopia in some but not all studies,[13-17] while genetic polymorphisms in vitamin D pathway genes have been associated in 1 study but not in another.[13,17]

We exploited the availability of relevant existing information (ie, refractive status, UVR, education, serum vitamin D concentrations, and genetic polymorphisms in vitamin D pathway genes) in the European Eye Study with the objective of investigating their association with myopia.

Key Points

Question What is the association between myopia and ultraviolet B radiation, serum vitamin D concentrations, and polymorphisms in vitamin D metabolism genes in a cross-sectional, population-based random sample of participants 65 years and older from north and south Europe?

Findings In this secondary analysis of the European Eye Study, only ultraviolet B radiation exposure was associated with a reduced odds ratio for myopia, especially in adolescence and early adulthood, despite adjustment for years in education.

Meaning This study, while not designed to determine cause and effect relationships, suggests that increased ultraviolet B exposure, a marker of sunlight exposure, is associated with reduced myopia.

Methods

Study Population

The European Eye Study was designed to maximize heterogeneity of UVR exposure and diet by selection of study centers from northern to southern Europe. Participants were recruited from November 6, 2000, to November 15, 2002, from random sampling of the population 65 years and older in the following centers: Bergen, Norway; Tallinn, Estonia; Belfast, United Kingdom; Paris-Creteil, France; Verona, Italy; Thessaloniki, Greece; and Alicante, Spain.[18] More than 11 000 people were invited, of whom 5040 participated (45.8% response rate). Written informed consent was obtained from all study participants. Ethical approval was obtained for each center from the local ethics committee, and the research adhered to the tenets of the Declaration of Helsinki.

Details of study design are described elsewhere.[19] Participants attended the examination center where they were interviewed by trained fieldworkers, underwent an ophthalmological examination, and gave a blood sample for blood measurements and genotyping. Information collected by the interviewers included years of education, smoking, alcohol use, a brief medical history, a semiquantitative food frequency questionnaire, and a detailed questionnaire on outdoor exposure.

Measurement of UV Exposure

Full details of the methods have been published previously.[20] Participants were sent a residence and employment history survey to complete in advance to facilitate recall at the interview. We used a questionnaire that asked about time spent outdoors between the hours of 9 AM and 5 PM and between 11 AM and 3 PM daily (from the age of 14 years) for different occupational and leisure periods (including homecare) and in retirement up to current age. Information from the questionnaire and residence calendar and geographical coordinates for residence were sent to the University of East Anglia in the United Kingdom to generate estimates of individual years of all-day (9 AM to 5 PM) or middle-of-the-day (11 AM and 3 PM) exposure for different wavelengths of light (ultraviolet A, ultraviolet B [UVB], and blue light). For all residences of 1 year or more, ambient UVB (minimal erythema dose[21]) and ultraviolet A (J/cm^2) were estimated from published sources that take into account time of day, month, and latitudinal variations.[22] We used published coefficients to adjust ambient clear-sky UV for cloud cover[23] and terrain.[24] For each wavelength of light, maximum potential lifetime dose was calculated as the sum of the time-weighted levels at each of the places of residence of the individual. Personal adult lifetime (ie, from age 14 years) UV exposure was estimated for each of the 3 wavelengths and summed for a mean annual lifetime dose at different ages for all-day and middle-of-the-day exposure.

Visual Acuity and Refraction

The protocol for testing visual acuity (VA) was different in 1 of the European Eye Study centers (Alicante, Spain); data from this center was not included in the present analysis. All other centers followed the procedures described below. Presenting distance VA (ie, with spectacles if worn) was tested separately in each eye using the 4-meter ETDRS logMAR chart. Any participant who was unable to achieve 0.3 logMAR (ie, a 20/40 Snellen acuity) in either eye underwent automated refraction or manual retinoscopy, and their best-corrected VA was recorded. For persons who achieved 0.3 logMAR or better, the spectacle correction (if any) worn by the participant for each eye was measured by neutralization using a focimeter or by handheld lenses. The spherical equivalent was obtained by

adding half of the cylindrical value to the spherical value and the mean of the 2 eyes was calculated, commonly used in epidemiological studies. Myopia was defined as a spherical equivalent of −0.75 diopters (D) or less (low myopia, ≤−0.75 to >−3 D; moderate myopia, ≤−3 to >−6 D; severe myopia, ≤−6 D). Those with a spherical equivalent greater than −0.75 D were not considered to have myopia, nor were those with an unaided VA higher than 0.3 logMAR when refraction was not measured. Participants with late age-related macular degeneration (AMD), aphakia or pseudophakia in either eye, or visual impairment (ie, less than 0.5 logMAR or 20/60 Snellen acuity or less) due to cataract were excluded.

Blood Measurement

Blood samples were sent to a single laboratory (Queen's University Belfast in the United Kingdom) for analysis. Serum 25-hydroxy vitamin D_2 (25[OH]D_2) and 25-hydroxy vitamin D_3 (25[OH]D_3) concentrations were measured by liquid chromatography-tandem mass spectrometry.[25] In all analyses, vitamin D levels were adjusted for season of measurement. Plasma lutein concentrations, zeaxanthin concentrations, β-cryptoxanthin concentrations, α-carotene and β-carotene concentrations, α-tocopherol and γ-tocopherol concentrations, lycopene concentrations, and retinol concentrations were measured by reversed-phase high-performance liquid chromatography. Total ascorbate was measured using an enzyme-based assay in plasma stabilized with metaphosphoric acid. All assays were standardized against appropriate National Institute of Standards and Technology standard reference materials. Cholesterol was measured using an enzymatic assay (Randox, Crumlin) on a Cobas FARA centrifugal analyzer (Roche Diagnostics).

Statistical Analysis

Statistical analysis was carried out using Stata version 13 (StataCorp). All analyses took account of the study design of the 6 centers by use of robust errors. All-day (9 AM to 5 PM) adult lifetime UVB exposure and 25(OH)D_3 concentrations were the primary measures of interest, as vitamin D_3 is produced in the skin following exposure to UVB whereas vitamin D_2 is mainly derived from fortified foods and vitamin supplements.[26] Following the exclusion of 67 participants with very high levels, the distribution of 25(OH)D_3 concentrations was normal. We investigated 25(OH)D_3 both as a continuous variable and categorized by quintiles. Dietary vitamin D was estimated using food composition tables[27] and was energy adjusted. Exposure to UVB was normalized using a square root transformation and then *z* transformed to investigate an increase in exposure of 1 SD. We calculated years of education from the difference between the start and leaving dates and categorized these data into tertiles to reflect the common tiers of education (ie, primary, secondary, and higher) for inclusion as an independent myopia risk factor.

We ran preliminary regression analyses to identify factors associated with changes in 25(OH)D_3 concentrations and with UVB as possible confounders of any association with myopia. A large number of variables were independently associated with 25(OH)D_3 concentrations, including age, sex, season, study center, current smoking, diabetes, obesity, dietary vitamin D intake, fish and fish oil supplement intake, and antioxidants, including vitamin C, lutein (or zeaxanthin), retinol, α-tocopherol, and cholesterol. Lutein and zeaxanthin were highly correlated (r = 0.85), and results were almost identical when separately introduced into the models; we presented lutein only for simplicity. Of these, only lutein was (inversely) associated with myopia and entered the models as a potential confounder. The factors independently associated with UVB were 25(OH)D_3 concentrations, study center, sex, and education; only education was (positively) associated with myopia. Therefore, in our final logistic regression models for myopia, we retained age, sex, study center, and season as well as our primary exposure variables (UVB, 25[OH]D_3, and education) and identified confounders, namely lutein. Our outcome measure was the confounder-adjusted association between myopia and our key exposures expressed as the adjusted odds ratio (OR) in logistic regression.

Single-Nucleotide Polymorphism Selection, Genotyping, and Genetic Analyses

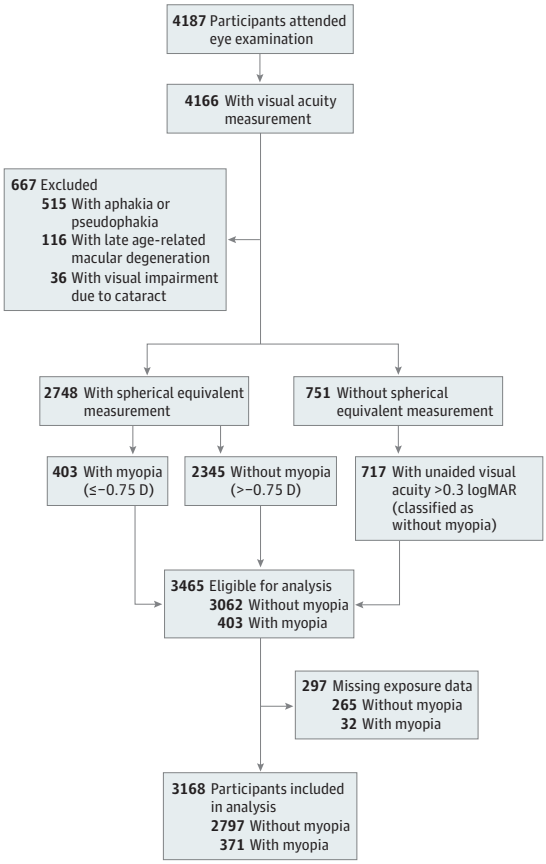
For reason of costs, genotyping was undertaken in a subsample of the main study. Data on vitamin D pathway single-nucleotide polymorphisms (SNPs) were available for a subset of 109 of 371 participants (29.4%) with myopia and 782 of 2797 participants (28.0%) without myopia. Ninety-three common SNPs located across 7 genes involved in vitamin D metabolism—*GC* (10), *RXRA* (14), *CYP2R1* (7), *DHCR7* (5), *VDR* (29), *CYP27B1* (7), and *CYP24A1* (21)—were selected from Phase III, release 2 HapMap data of Utah residents with ancestry in northern and western Europe using Haploview (http://www.broadinstitute.org/haploview) to determine linkage disequilibrium. Tag SNPs were selected using multimarker tagging with the following criteria: r^2 greater than 0.8, minor allele frequency of 5% or greater, genotype call rate of 95% or greater, and no significant deviation from Hardy Weinberg equilibrium. Genotyping was performed by KBiosciences, and associations between genotypes and myopia status were investigated. Quality filters for exclusion of SNPs included call rates less than 95% and deviation from Hardy Weinberg equilibrium (P < .001). DNA samples were excluded if missing genotypes exceeded 10%. Other quality control measures included duplicates on plates, random sample allocation to plates, independent scoring of problematic genotypes by 2 individuals, and resequencing selected DNAs to validate genotypes. KBiosciences quality control also included validation of all SNP assays on a panel of 44 random white participant-derived samples and 4 nontemplate (negative) controls. Statistical genetic tests were performed using PLINK version 1.07 under an additive genotypic model.[28] Logistic regression adjusted for age, sex, season, and study center to examine association with individual SNPs.

Results

The flow of participants in the study design is illustrated in **Figure 1**. We excluded 515 participants for aphakia or pseudo-

phakia, 116 for late AMD, and 36 for vision impairment due to cataract. Relevant exposure data (mainly serum 25[OH]D_3 concentrations) were missing in 297 participants (32 with myopia and 265 without myopia). Our final analysis was based on 371 participants with myopia, of which 24 (6.5%) had high myopia, and 2797 without myopia with complete data on all relevant exposures. Included participants had a mean (SD) age of 72.4 (5) years, and 1456 (46.0%) were male.

Figure 1. PRISMA Flowchart of Inclusion of Study Participants



In univariate analyses, there were no differences in the age or sex of people with myopia compared with those without, nor in smoking habit, alcohol use, or obesity (**Table 1**). Significant differences were observed between those with and without myopia in years of education, UVB exposure, and serum 25(OH)D_3 concentrations, but there was no difference in dietary vitamin D intake.

In analyses adjusted for age, sex, and study center, an increase of 1 SD in personal lifetime UVB exposure was associated with reduced odds of myopia (OR, 0.72; 95% CI, 0.56-0.93; P = .001) (**Table 2**). Those in the highest tertile of years of education (median, 14 years) had twice the odds of myopia (OR, 2.08; 95% CI, 1.41-3.06; P = .001) compared with those in the lowest tertile (median, 7 years). In the adjusted analyses, there was no clear evidence for an association of 25(OH)D_3 concentrations (either continuous or by quintiles) with myopia. In contrast, those in the highest quintile of plasma lutein concentrations had nearly half the risk of myopia (adjusted OR, 0.57; 95% CI, 0.46-0.72) compared with the lowest quintile. In a further model adjusted for age, sex, study center, and season and incorporating 25(OH)D_3 concentrations, lutein concentrations, education, and UVB, the estimates for each exposure were virtually unchanged. There was evidence for a stronger inverse association of UVB with increasing myopia severity (low myopia: OR, 0.87; 95% CI, 0.75-1.01; P = .06; moderate myopia: OR, 0.59; 95% CI, 0.36-0.97; P = .04; severe myopia: OR, 0.39; 95% CI, 0.25-0.63; P = .001).

We investigated whether the association with myopia and UVB exposure varied by the personal UVB exposure experienced at different ages. Significant ORs for less myopia with increased UVB exposure were observed in adolescence and early adulthood, between ages 14 to 19 years and 20 to 29 years (**Figure 2**), but not for other age groups.

The subset of 891 patients (28.1%) with genetic data were similar in age (mean [SD] age, 73 [5] years), sex (49% male), and myopia severity (low myopia, 59%; moderate, 34%; and high, 7%) to those without genetic data. Of the 93 genetic variants associated with vitamin D metabolism, 1 SNP in *GC* was excluded for deviation from Hardy Weinberg equilibrium. Of the remaining SNPs, 4 were nominally associated with myo-

Table 1. Characteristics of Participants With and Without Myopia

Characteristic[a]	Myopia (n = 371)	Without Myopia (n = 2797)	*P* Value[b]
Age, mean (SD), y	72.9 (5.5)	72.4 (5.0)	.58
Male, No. (%)	174 (46.9)	1282 (45.8)	.83
Years of education, median (IQR)	11 (7-14)	9 (7-12)	.01
UVB (minimal erythema dose), median (IQR)[c]	314 (140-566)	358 (224-585)	.01
25(OH)D_3, mean (SD), nmol/L	45.3 (20.8)	47.5 (20.9)	.01
Dietary vitamin D, median (IQR), µg/d	1.86 (1.32-2.62)	1.89 (1.35-2.56)	.62
Ever smoked, No. (%)	179 (48.2)	1350 (48.3)	.98
Alcohol at least weekly, No. (%)	134 (36.1)	1106 (39.5)	.49
Obesity (BMI >30), No. (%)	138 (37.2)	1001 (35.8)	.82
Lutein, median (IQR), µmol/L	0.087 (0.04-0.24)	0.130 (0.05-0.39)	<.01

Abbreviations: 25(OH)D_3, serum 25-hydroxy vitamin D_3; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); IQR, interquartile range; UVB, ultraviolet B radiation.

[a] Univariate analyses.

[b] Difference in characteristic between those with and without myopia.

[c] Mean annual UVB exposure.

Table 2. Association of Ultraviolet B Radiation Exposure, Education, Serum Vitamin D_3 Concentrations, and Lutein Concentrations With Myopia

Characteristic	Adjusted OR (95% CI)[a]	*P* Value[b]	Adjusted OR (95% CI)[c]	*P* Value[b]
UVB exposure (1 SD increase)	0.72 (0.56-0.93)	.01	0.75 (0.58-0.97)	.03
Years of education, median		.001		<.001
First tertile (7)	1 [Reference]	NA	1 [Reference]	NA
Second tertile (10)	1.26 (0.99-1.58)	.06	1.22 (0.96-1.57)	.10
Third tertile (14)	2.08 (1.41-3.06)	.001	2.04 (1.40-2.96)	.001
25(OH)D_3 concentrations (continuous)	0.99 (0.98-1.00)	.48	NA	NA
Quintiles of 25(OH)D_3, median, nmol/L		.31		.31
First quintile (19.9)	1 [Reference]	NA	1 [Reference]	NA
Second quintile (33.1)	0.96 (0.79-1.31)	.78	0.95 (0.74-1.22)	.77
Third quintile (45.3)	0.87 (0.64-1.38)	.55	0.89 (0.59-1.36)	.62
Fourth quintile (58.9)	0.75 (0.47-1.20)	.24	0.78 (0.51-1.20)	.28
Fifth quintile (77.0)	0.87 (0.51-1.47)	.60	0.87 (0.56-1.38)	.59
Quintiles of plasma lutein, median, µmol/L		<.001		<.001
First quintile (0.03)	1 [Reference]	NA	1 [Reference]	NA
Second quintile (0.05)	0.93 (0.80-1.08)	.34	0.94 (0.81-1.10)	.48
Third quintile (0.11)	0.82 (0.55-1.20)	.30	0.83 (0.55-1.25)	.39
Fourth quintile (0.22)	0.89 (0.62-1.27)	.51	0.87 (0.63-1.19)	.41
Fifth quintile (0.48)	0.57 (0.46-0.72)	.001	0.59 (0.48-0.73)	<.001

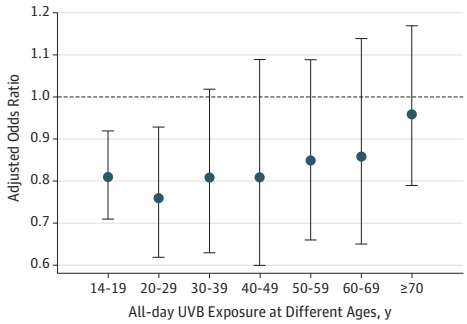
Abbreviations: 25(OH)D_3, serum 25-hydroxy vitamin D_3; NA, not applicable; OR, odds ratio; UVB, ultraviolet B radiation.

[a] Adjusted for age, sex, study center, and season for 25(OH)D_3 and lutein concentrations.

[b] *P* value for effect of each variable on myopia.

[c] Adjusted for age, sex, study center, season, and all variables in the model (namely, UVB exposure, education, 25(OH)D_3 concentrations, and plasma lutein concentrations).

Figure 2. Association of All-Day Ultraviolet B (UVB) Exposure at Different Ages With Myopia



Adjusted for age at time of examination, sex, study center, and years of education. Error bars indicate 95% CIs.

pia (3 in *CYP2RI* and 1 in *CYP24A1*), but none withstood correction for multiple testing (eTable in the Supplement).

Discussion

We found that higher annual lifetime UVB exposure, directly related to time outdoors and sunlight exposure, was associated with reduced odds of myopia. Exposure to UVB between ages 14 and 29 years was associated with the highest reduction in odds of adult myopia. Myopia was more than twice as common in participants in the highest tertile of education. The association between UVB, education, and myopia remained even after respective adjustment. This suggests that the high rate of myopia associated with educational attainment is not solely mediated by lack of time outdoors.

The protective effect of time outdoors on myopia is well established.[6-9,29] Time outdoors reflects various physiological effects that have been associated with or hypothesized to influence myopia, including brighter light levels,[30,31] a different spectrum of wavelengths compared with artificial lighting with reduced UVR, and an extended focal distance with less hyperopic peripheral defocus.[32] Ultraviolet conjunctival autofluorescence, an indirect marker of ocular sun exposure (in particular, UVR), is inversely associated with myopia[8] and has a stronger effect than time outdoors assessed using questionnaires. One small study[33] measuring UVR using dosimeters found differing exposure between those with emmetropia, those with stable myopia, and those with progressing myopia.

Proposed mediating mechanisms include activation of dopaminergic retinal amacrine cells, which are stimulated by light[31] and influence ocular axial growth,[34] and higher serum vitamin D concentrations induced by sunlight. We, like others, did not find evidence to support the association between myopia and serum vitamin D concentrations[16] or genes involved in vitamin D metabolism. A previous publication[17] examined 12 SNPS from 2 vitamin D pathway genes (*VDR* and *GC*) and reported a significant association between rs2853559 in *VDR* in the overall sample of 289 participants with myopia and 81 controls and a further 3 variants in *VDR* within a subset of participants with low and moderate myopia. In a more recent publication,[13] 33 SNPs across 6 genes associated with vitamin D metabolism were examined in more than 2000 individuals in relation to both refractive error and axial length. Nominal significance was identified for variants in *CYP24A1* and *VDR*, but none withstood correction for multiple testing. We inves-

tigated the association between myopia and 92 variants in vitamin D metabolism genes, identifying nominal significance in 3 SNPs in *CYP2R1* and 1 SNP in *CYP24A1* (not the same variant as the aforementioned study). None withstood correction for multiple testing. We acknowledge low power for this type of analysis, but notably, we studied more variants as well as previously unexamined genes (ie, *CYP2R1* and *RXRA*) in a substantial cohort.

Those in the highest fifth of plasma lutein concentrations had approximately 40% reduced odds of myopia. We excluded those with late AMD because we have previously shown an increased risk of late AMD with blue light exposure in those with low levels of key antioxidants, including lutein.[20] Sensitivity analyses made no appreciable difference; myopia (OR, 0.56; 95% CI, 0.46-0.70) in the highest quintile of lutein was similar when 72 individuals with late AMD were included or excluded (OR, 0.57 vs 0.56). Lutein is a retinal carotenoid, responsible for much of the macular pigment optical density, and has antioxidative, anti-inflammatory, and structural effects in neural tissue.[35] Lutein has been associated with a reduced risk of AMD,[36] with improved contrast sensitivity in healthy individuals,[37] and (inversely) with axial length (and thus axial myopia).[38] Although limited evidence for an association between lutein and myopia is gained from this analysis and, importantly, no causative role can be inferred, it does raise interesting hypotheses for a potential role.

Study Limitations

This study has limitations. We retrospectively calculated UVB exposure data through highly detailed questionnaires over the life course and used this data together with geographically specific, historical data on UVR. Our measure is subject to recall error and lacks the heightened accuracy of UV exposure achieved with light meters. However, we do not have any reason to believe that the UVB association would be biased, as myopia was identified after the interview. A weakness of our study was that we did not collect any data on UVB exposure during childhood, which could be argued to be more relevant in myopia development. However, a significant proportion of refractive error develops in adolescence and early adulthood,[39] and our results showed the greatest effects for these age groups. No myopia was defined either by refraction or good, unaided VA when refraction was unknown. This definition was used in attempt to minimize bias, but to ensure this was appropriate, we performed sensitivity analyses in which those without myopia were only classified on the basis of measured refractive error; analysis using this definition produced very similar results. A limitation was also that vitamin D and lutein concentrations were measured in later life. The association between myopia development and these factors may be more relevant in younger ages. However, there is evidence, albeit limited, that an individual's 25(OH)D concentrations are reproducible over time.[40] Variants in vitamin D pathway genes are not subject to these concerns of temporality and confounding (mendelian randomization); hence, any association with myopia would strengthen a causal relationship with vitamin D. Therefore, we consider it unlikely that vitamin D plays a role in myopia.

Conclusions

This study suggests lifetime exposure of UVB is associated with reduced myopia in adulthood. The protective association is strongest with exposure in adolescence and younger adult life and with increasing severity of myopia. As the protective effect of time spent outdoors is increasingly used in clinical interventions, a greater understanding of the mechanisms and life stages at which benefit is conferred is warranted.

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Supplementary Online Content

Williams KM, Bentham GCG, Young IS, et al. Association between myopia, ultraviolet B radiation exposure, serum vitamin D concentrations, and genetic polymorphisms in vitamin D metabolic pathways in a multicountry European study. *JAMA Ophthalmol*. Published online December 1, 2016. doi:10.1001/jamaophthalmol.2016.4752.

eTable. Association between myopia and genetic variants associated with vitamin D metabolic pathway

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable. Association between myopia and genetic variants associated with vitamin D metabolic pathway

Chromosome	SNP	Gene	Numbers tested	OR	95% CI	P*
11	rs7935792	*CYP2R1*	872	2.17	1.28 - 3.68	0.004
11	rs7117967	*CYP2R1*	870	1.71	1.05 - 2.79	0.030
11	rs10832306	*CYP2R1*	877	1.58	1.02 - 2.45	0.043
20	rs6097809	*CYP24A1*	867	0.45	0.20 - 0.99	0.048
11	rs4316537	*DHCR7*	862	0.51	0.24 - 1.09	0.083
12	rs2269720	*VDR*	871	0.76	0.54 - 1.07	0.113
4	rs1565572	*GC*	872	1.31	0.93 - 1.85	0.121
12	rs11574143	*VDR*	862	0.66	0.39 - 1.13	0.130
12	rs1021469	*VDR*	873	0.78	0.56 - 1.09	0.143
4	rs705117	*GC*	878	1.33	0.89 - 1.98	0.170
12	rs4760655	*VDR*	874	0.80	0.58 - 1.10	0.173
12	rs11574077	*VDR*	874	0.55	0.23 - 1.31	0.178
4	rs16847050	*GC*	868	1.30	0.86 - 1.94	0.210
9	rs10881582	*RXRA*	874	1.24	0.88 - 1.73	0.214
12	rs4760648	*VDR*	875	0.82	0.61 - 1.12	0.215
11	rs12785878	*DHCR7*	874	0.81	0.58 - 1.13	0.217
9	rs11185659	*RXRA*	872	1.24	0.88 - 1.74	0.225
20	rs2762932	*CYP24A1*	874	1.27	0.86 - 1.86	0.226
20	rs2762941	*CYP24A1*	871	1.21	0.89 - 1.65	0.232
12	rs886441	*VDR*	874	1.24	0.87 - 1.78	0.234
4	rs222020	*GC*	873	1.27	0.85 - 1.90	0.242
12	rs11574027	*VDR*	874	1.75	0.67 - 4.58	0.252
12	rs4646537	*VDR*	878	1.52	0.73 - 3.17	0.259
12	rs11168302	*VDR*	876	1.67	0.68 - 4.05	0.261
4	rs2298850	*GC*	853	0.82	0.57 - 1.17	0.264
9	rs12339187	*RXRA*	868	1.22	0.85 - 1.76	0.287
11	rs1792284	*CYP2R1*	863	0.83	0.59 - 1.17	0.289
20	rs6127119	*CYP24A1*	873	1.20	0.84 - 1.69	0.314
4	rs1491718	*GC*	877	1.26	0.80 - 1.98	0.323
9	rs914853	*RXRA*	875	1.17	0.85 - 1.61	0.328
12	rs2853564	*VDR*	871	1.16	0.85 - 1.58	0.338
12	rs2254210	*VDR*	873	1.16	0.86 - 1.56	0.340
12	rs2239186	*VDR*	875	1.17	0.84 - 1.64	0.353
12	rs10877011	*VDR*	873	0.86	0.62 - 1.18	0.355
12	rs4760169	*VDR*	871	1.24	0.78 - 1.96	0.362
12	rs12368653	*VDR*	878	0.89	0.66 - 1.19	0.429
20	rs2181874	*CYP24A1*	876	1.15	0.82 - 1.60	0.430
12	rs3819545	*VDR*	876	1.13	0.83 - 1.53	0.441

12	rs7975232	*VDR*	874	1.12	0.84 - 1.49	0.448
9	rs6537944	*RXRA*	875	1.23	0.70 - 2.16	0.464
20	rs2585428	*CYP24A1*	872	0.89	0.66 - 1.21	0.470
12	rs731236	*VDR*	870	1.12	0.82 - 1.51	0.480
4	rs1155563	*GC*	862	0.88	0.62 - 1.26	0.486
12	rs12717991	*VDR*	876	0.90	0.68 - 1.21	0.489
11	rs949178	*DHCR7*	877	0.81	0.45 - 1.48	0.498
9	rs11185644	*RXRA*	869	0.87	0.57 - 1.32	0.503
12	rs2239179	*VDR*	871	0.91	0.67 - 1.22	0.511
9	rs4842196	*RXRA*	876	1.12	0.80 - 1.57	0.514
12	rs2239182	*VDR*	875	0.91	0.68 - 1.22	0.516
20	rs2245153	*CYP24A1*	874	1.13	0.78 - 1.64	0.529
12	rs2238136	*VDR*	875	1.11	0.80 - 1.53	0.548
4	rs11939173	*GC*	871	1.08	0.80 - 1.46	0.597
20	rs6068816	*CYP24A1*	867	0.87	0.51 - 1.48	0.605
20	rs2296241	*CYP24A1*	875	1.08	0.80 - 1.45	0.619
12	rs7136534	*VDR*	864	1.09	0.76 - 1.55	0.641
12	rs11168268	*VDR*	875	1.07	0.80 - 1.44	0.659
12	rs1544410	*VDR*	875	1.07	0.79 - 1.45	0.669
20	rs11907350	*CYP24A1*	872	0.83	0.35 - 1.97	0.671
11	rs1496167	*CYP2R1*	870	0.93	0.68 - 1.29	0.674
12	rs1048691	*VDR*	874	1.08	0.75 - 1.56	0.674
12	rs10875702	*VDR*	873	1.08	0.74 - 1.59	0.685
20	rs2248359	*CYP24A1*	870	1.07	0.78 - 1.45	0.687
20	rs1570669	*CYP24A1*	873	0.94	0.69 - 1.28	0.706
12	rs10875693	*VDR*	874	0.94	0.69 - 1.29	0.719
9	rs11103482	*RXRA*	867	0.91	0.56 - 1.50	0.720
11	rs12419657	*CYP2R1*	877	0.92	0.58 - 1.48	0.738
9	rs3118526	*RXRA*	876	1.08	0.69 - 1.69	0.742
12	rs2107301	*VDR*	868	1.05	0.77 - 1.44	0.757
9	rs4240705	*RXRA*	874	1.05	0.77 - 1.43	0.766
12	rs6580642	*VDR*	874	1.06	0.71 - 1.58	0.791
9	rs3118571	*RXRA*	873	0.96	0.71 - 1.30	0.791
9	rs11103473	*RXRA*	875	0.96	0.71 - 1.32	0.818
20	rs4809960	*CYP24A1*	870	0.96	0.67 - 1.38	0.837
11	rs7950649	*DHCR7*	869	0.96	0.61 - 1.51	0.852
11	rs11023371	*CYP2R1*	862	1.06	0.58 - 1.92	0.861
20	rs6068810	*CYP24A1*	869	0.94	0.46 - 1.91	0.862
12	rs4516035	*VDR*	875	1.03	0.75 - 1.40	0.870
20	rs4809959	*CYP24A1*	877	1.02	0.76 - 1.37	0.892
12	rs2283342	*VDR*	863	0.98	0.67 - 1.43	0.896
9	rs7039190	*RXRA*	868	0.95	0.46 - 2.00	0.901
20	rs3787557	*CYP24A1*	873	1.03	0.67 - 1.58	0.902
9	rs3118536	*RXRA*	868	0.98	0.66 - 1.44	0.904
20	rs3787555	*CYP24A1*	876	0.98	0.70 - 1.37	0.904

12	rs11574026	*VDR*	874	1.02	0.63 - 1.66	0.937
20	rs6022999	*CYP24A1*	871	1.01	0.71 - 1.44	0.937
11	rs1037379	*CYP2R1*	864	1.01	0.73 - 1.40	0.943
12	rs11574024	*VDR*	872	0.99	0.59 - 1.64	0.961
20	rs927650	*CYP24A1*	871	0.99	0.74 - 1.34	0.968
20	rs2274130	*CYP24A1*	866	1.00	0.70 - 1.42	0.982
4	rs12512631	*GC*	878	1.00	0.74 - 1.37	0.983
12	rs2189480	*VDR*	869	1.00	0.74 - 1.35	0.992
20	rs927651	*CYP24A1*	872	1.00	0.71 - 1.42	0.992

*No adjustment for multiple testing.
SNP = single nucleotide polymorphism, OR = odds ratio, 95% CI = 95% confidence interval, p = p value.

Chapter 5 | Genetic factors for myopia and the interplay with environmental risk factors

5.1 Introduction

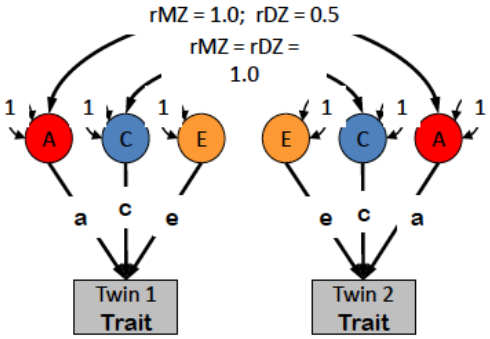
In this chapter I firstly present a classical twin study and genome-wide association study (GWAS) in TEDS. Performing classical twin analyses became a major part of my PhD, with many collaborations formed where I performed heritability analysis in other ocular phenotypes resulting in publications (258-260). Whilst not novel, I here detail the methods and findings of a heritability estimate for refractive error using the classical twin model in TEDS. Secondly, I present the methodology and results of a GWAS for refractive error in TEDS. Whilst I am very much underpowered to detect novel variants, I became familiar with the methodology, which was useful for subsequent work, and I also discuss the relative effect of genetic variants identified for refractive error from adult GWAS. I next present manuscripts (one in review and one accepted); in the first I explore the genetic correlation between myopia and intelligence using bivariate twin modeling and molecular genetic data in the form of polygenic risk scores. The final publication is a gene by environment (GxE) analysis concerning the interaction between myopia and two known associations, near work and time outdoors. Very large sample sizes are required for GxE analyses and for this work I collaborated with other childhood cohorts in the Consortium for Refractive Error and Myopia (CREAM) where I performed analyses for the TEDS study.

5.2 Heritability and associated genetic variants for refractive error in TEDS

5. 21 Classical twin study in TEDS

5.211 Methods

Heritability was calculated using maximum likelihood, structural-equation classical twin modeling with the OpenMx (261) package in R. In this technique the variance of a trait is estimated by the contributions of three latent factors: the additive genetic component (A), the shared environment (C) or the dominant genetic component (D), and the unique environment (E). Path diagrams are constructed to map the variance of each trait in terms of ACE/ADE and the covariance between these latent factors in each twin. In the twin scenario there are constant relationships such as complete sharing of the shared home environment, and additive genetic correlation of 1.0 in MZ twins and 0.5 in DZ twins. Graphical illustrations of these covariances and variances are converted to matrices for analysis in the OpenMx package [Figure 4.1].



MZ	Twin 1	Twin 2
Twin 1	$a^2 + c^2 + e^2$	$a^2 + c^2$
Twin 2	$a^2 + c^2$	$a^2 + c^2 + e^2$

DZ	Twin 1	Twin 2
Twin 1	$a^2 + c^2 + e^2$	$0.5a^2 + c^2$
Twin 2	$0.5a^2 + c^2$	$a^2 + c^2 + e^2$

Figure 5.1 Path diagram and matrices for the variance and covariance of a trait in a twin pair (adapted from MRC Social, Genetic and Developmental Psychiatry Summer School 2013 Handbook)

In OpenMx an ACE and ADE model with standardized path coefficients and expected variance and covariance matrices is executed. The goodness of fit of the full and reduced model to the observed data is evaluated using minus twice log-likelihood (-2LL) and x^2 tests. A significant change in x^2 between the full model and reduced model indicates that the removed factor was not significant and could be dropped. Heritability is the proportion of total variance (V) of the trait (ie. $a^2 + c^2 + e^2$ or $a^2 + d^2 + e^2$) due to additive genetic effect (A) or additive plus dominant genetic effect (A + D), which can be calculated using the formula "h2 (heritability) = A/V or A+D/V". All quantitative measures were adjusted for age and sex.

5.212 Results

Those in whom refractive data was only available on one twin were excluded from this element of the study. After exclusions refractive data was available for 333 MZ twin pairs and 579 DZ pairs. Spherical equivalent was highly correlated for MZ pairs (r 0.85, $p<0.001$) and displayed poor correlation in DZ pairs (r 0.30, $p<0.001$).

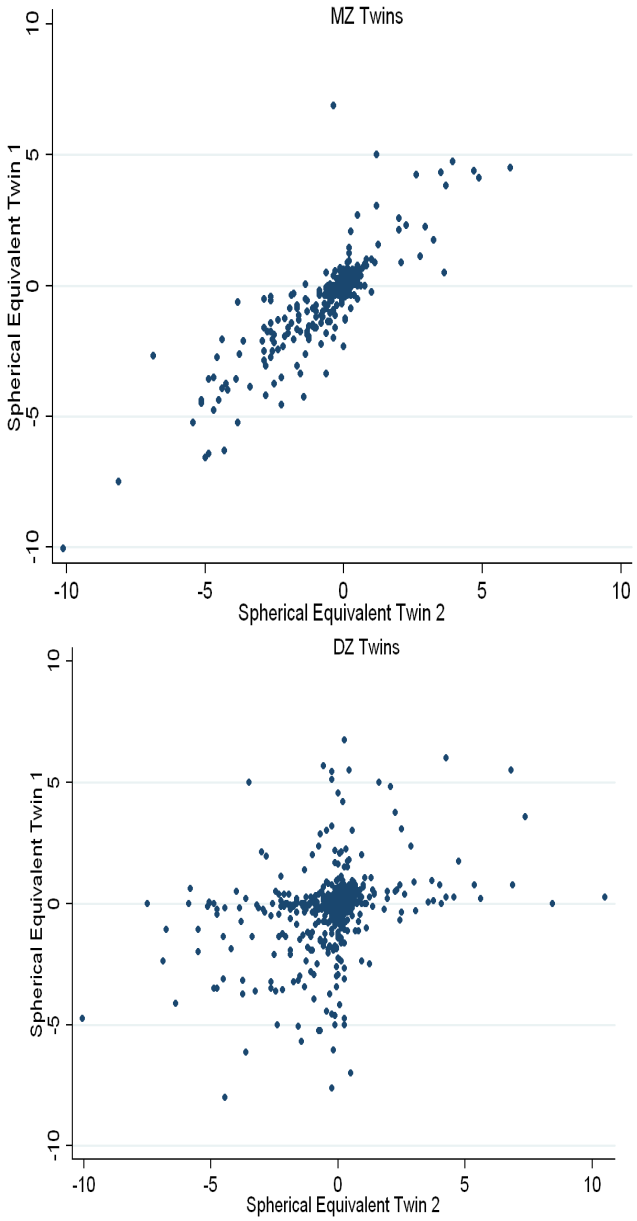


Figure 5.2 Scatter plots of spherical equivalent for MZ and DZ twin pairs

A univariant ACE/ADE twin model was performed in OpenMx on 333 MZ twin pairs and 579 DZ pairs. The best-fit model to explain the observed variance in spherical equivalent was the ADE model; as shown in the table below, when D was dropped from the ADE model there was a significant change in the fit. Another way to identify the best-fit model is to ascertain which model had the lowest Akaike information criterion (AIC).

Model	-2LL	df	AIC	diffLL	diffdf	p
ACE	7466.363	1987	3492.363	-	-	-
ADE	**7455.802**	**1987**	**3481.802**	**-10.56152**	**0**	**0.19**
AE	7466.363	1988	3490.363	-8.84029^{-10}	1	0.01
CE	7675.098	1988	3699.098	208.7348	1	0
E	7944.218	1988	3966.218	477.8552	2	0

Table 5.1 Model-fitting results for univariant analysis of spherical equivalent. The Akaike information criterion (AIC) is used to evaluate the most parsimonious model, which is highlighted in bold. For each model the minus 2 log-likelihood (minus2ll), degrees of freedom (df), difference in log-likelihood (difLL), and difference in degrees of freedom (diffdf) and p value for model comparison are given. A = additive genetic effects, D = dominant genetic effects, C = common environmental effects and E = unique environmental effects (and measurement error)

The spherical equivalent heritability estimate from the best-fit ADE model, a combination of additive and dominant genetic effects, was 85.6% (95% CI 82.6 – 87.7). Unique environmental effects were estimated to contribute 14.4% (95% CI 12.3 - 17.0) to the variance of spherical equivalent

5.213 Discussion

The heritability estimate using this model was 86%, indicating a strong contribution of genetic factors for spherical equivalent. Model fitting analyses suggested the contribution of shared environment was not significant and unique environmental factors explained just 14% of the variance of spherical equivalent. If the fit of the model is not taken into account and an ACE model is considered, point estimates of A, C & E are 85.3%, 2.9^{-12} % and 14.6% respectively; despite twin studies having a low power to detect shared environmental factors, the point estimate is still zero, meaning we found no shared (family) environmental effect. This finding is very comparable to that observed in TwinsUK; when heritability was calculated on a similar sample size (n = 1012) the estimate was 86% (95% CI 81 – 89%) for right eyes (252) and this reduced fractionally to 77% (95% CI 68 - 84%) when assessed in a later larger TwinsUK sample (n = 4602) (179).

5.22 Genome-wide Association Study in TEDS

5.221 Methods

Genotyping was performed on DNA samples from 3665 individuals (one twin per pair) on Affymetrix GeneChip 6.0 single nucleotide polymorphisms (SNP) genotyping arrays (Affymetrix, Santa Clara, CA, USA) using standard experimental protocols, as part of the WTCC2 project (262). Genotypes at untyped markers were imputed with HapMap phase II and III, and WTCCC2 controls using IMPUTE v2 software. A total of 3152 DNA samples (1446 males and 1706 females) survived quality control for relatedness, hetereozygosity, ancestry and hybridization intensity outliers. Principal component analyses have previously been performed on TEDS - using the Tracy-Widom tests on a subset of 100,000 quality-controlled SNPs, after removing SNPs in linkage disequilibrium (r^r>0.2), eight axes were identified with a $p<0.05$ (263).

Association analyses were performed using Plink v1.9 (264) on genotyped and imputed markers. A Hardy-Weinburg equilibrium cut-off of p=0.0001 was used to include SNPs. Linear regression models were performed for spherical equivalent with the following covariates: age, sex and eight principal components to control for population stratification.

5.223 Results

Genotype and phenotypic data was available for 698 singletons. There was no significant genetic inflation, as illustrated in the quantile-quantile (Q-Q) plot in Figure 5.3. This plot illustrates the fact that most of the values lie along the line with a gradient of 1 and therefore are consistent with the null hypothesis of no association. The small number of SNPs departing from the straight line represent the significant associations, of which a large number would not be expected.

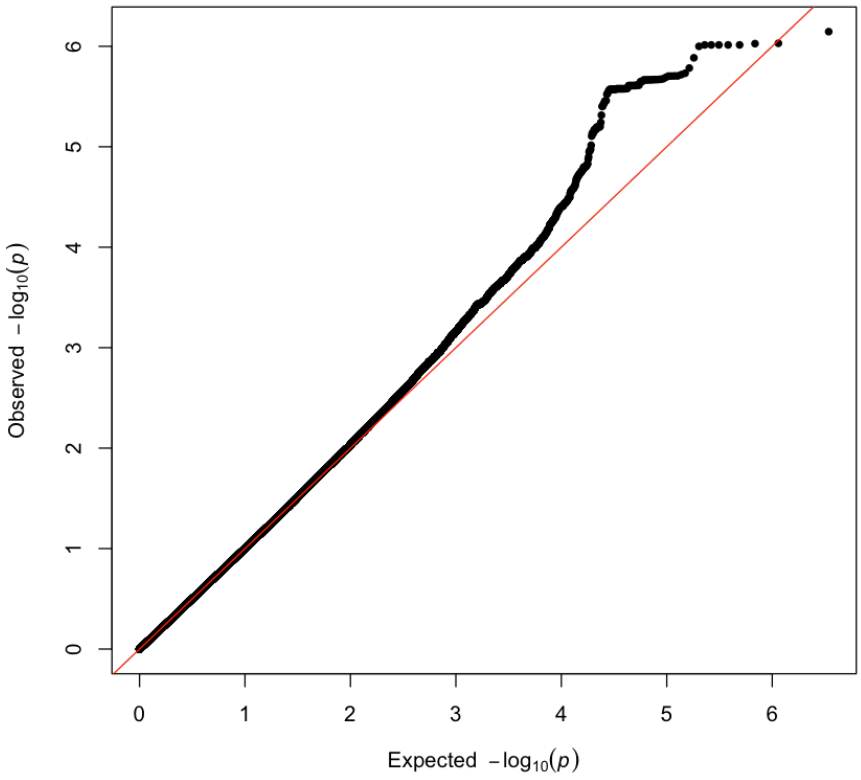


Figure 5.3 Q-Q plot for the association between all SNPs analysed and spherical equivalent in TEDS. Each dot represents an observed statistic (log10(P)) versus the corresponding expected statistic. The null distribution is illustrated by the red line.

The results of the GWAS are illustrated in the Manhattan plot in Figure 5.4. The strength of association between the SNPs and refractive error is on the y-axis, ordered by chromosome position on the x-axis. No SNPs cross the genome-wide significance level of $p < 5.0 \times 10^{-8}$. Ninety-one SNPs exceeded the suggestive significance threshold of $p = 1.0 \times 10^{-5}$. The SNPs were largely clustered on two genomic regions on chromosome 11 and 4. As illustrated in the regional plot in Figure 5.5, many loci on chromosome were in close proximity to olfactory genes. These are unlikely to be real associations and are likely the result of multiple testing.

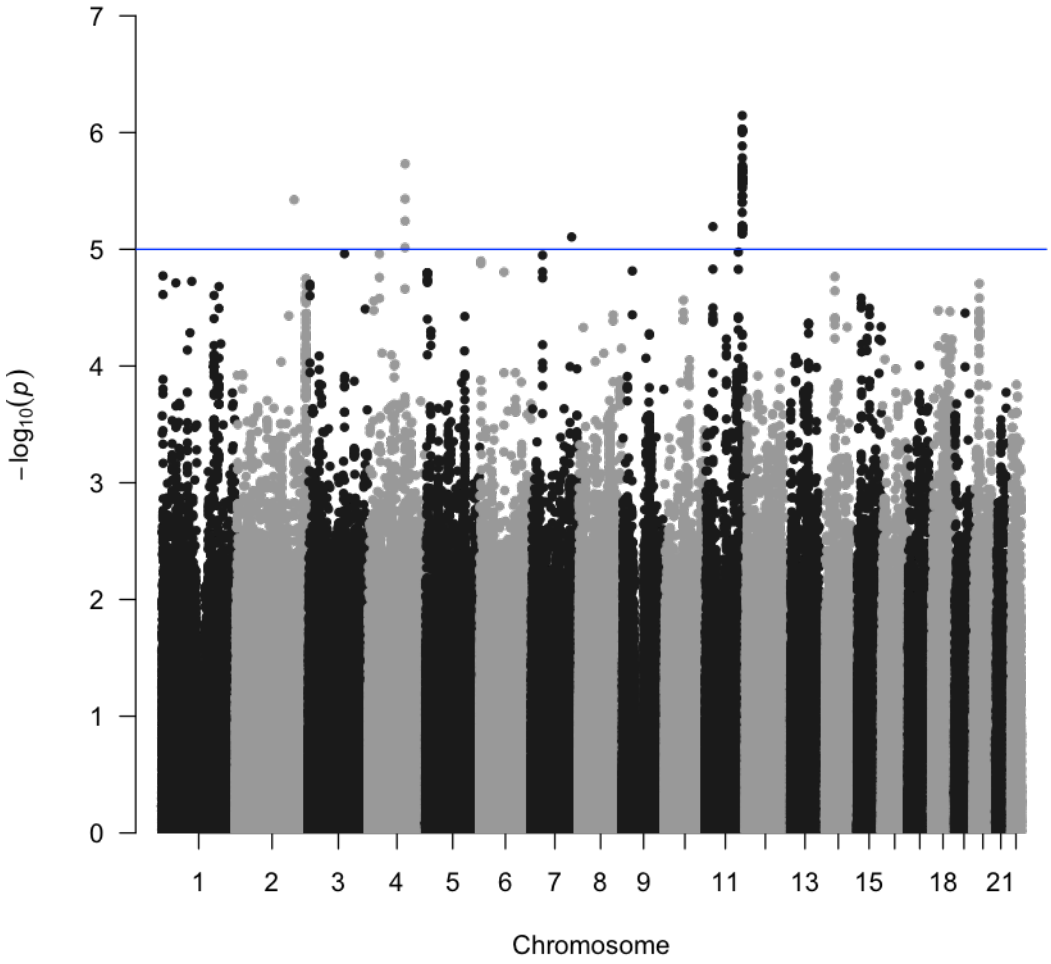


Figure 5.4 Manhattan plot of the GWAS for refractive error in TEDS (n=698). The y-axis is the $-\log_{10}(P)$ transformed P values for all SNPs. The blue line represents the suggestive significant threshold of p value = 1×10^{-5}.

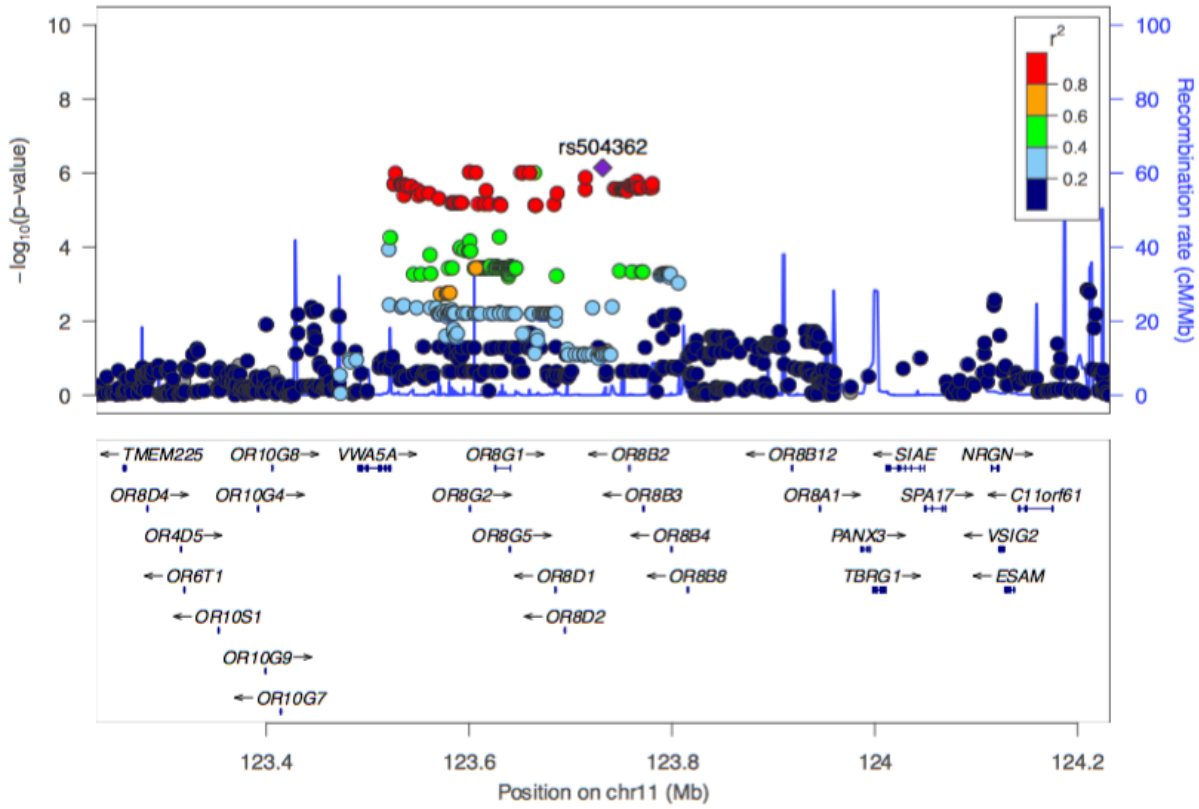


Figure 5.5 Regional plot of the loci associated on chromosome 11

The degree of association between the thirty-nine previously identified loci for refractive error from adult GWAS (265) were examined in TEDS. Twenty-eight loci were available for examination in TEDS following cleaning of genotyped and imputed SNPs. The results are illustrated in Table 5.2; only two loci were replicated at the p<0.05 threshold, *A2BP1* and *PCCA*. Interestingly A2BP1 (also called RBFOX1) was one of the most strongly associated loci in Europeans and the combined European and Asian analysis of childhood cohorts in CREAM (as described in Chapter 5.5).

CHR	SNP	gene	bp	A1	n	BETA	SE	P
16	rs17648524	*A2BP1*	7399684	C	675	-0.2268	0.09805	0.02104
13	rs2184971	*PCCA*	99616093	G	679	0.1869	0.09338	0.04571
17	rs17183295	*MYO1D*	28102385	T	691	0.2116	0.1258	0.09285
10	rs6480859	KCNMA1	78751954	T	691	0.1472	0.09108	0.1066
12	rs3138144	*RDH5*	54401036	C	689	0.1512	0.09485	0.1113
17	rs4793501	*KCNJ2*	66230329	C	679	0.1445	0.09193	0.1164
15	rs4778879	*RASGRF1*	77159930	G	674	-0.1378	0.09255	0.137
12	rs12229663	*PTPRR*	69536263	G	692	0.1609	0.1119	0.1509
2	rs17428076	DLX1	172560182	G	692	0.1499	0.1077	0.1643
11	rs2155413	DLG2	84312438	A	681	-0.1059	0.09019	0.2406
8	rs7829127	*ZMAT4*	40845551	G	692	0.117	0.1085	0.2813
9	rs11145465	*TJP2*	70956413	A	692	-0.09984	0.1084	0.3573
18	rs12971120	*CNDP2*	70325003	G	682	0.09664	0.105	0.3575
1	rs1652333	*PRSS56*	205537083	G	691	-0.08098	0.09707	0.4044
13	rs8000973	*ZIC2*	99489368	T	691	0.06984	0.08866	0.4312
8	rs4237036	*CHD7*	61863611	C	692	-0.06486	0.09442	0.4924
10	rs745480	RGR	85976534	G	683	-0.05634	0.08838	0.524
14	rs1254319	*SIX6*	59973510	A	666	0.06728	0.106	0.5259
10	rs7084402	*BICC1*	59935410	G	692	-0.05462	0.08918	0.5404
20	rs235770	*BMP2*	6709765	T	692	-0.05202	0.09082	0.567
15	rs634990	*GJD2*	32793365	C	618	-0.04266	0.09554	0.6554
3	rs13091182	ZBTB38	142616650	A	678	-0.03878	0.09578	0.6857
6	rs12205363	*LAMA2*	129876322	C	656	0.06765	0.1977	0.7324
8	rs7837791	*TOX*	60341640	T	578	-0.02546	0.09679	0.7926
2	rs1881492	*CHRNG*	233115242	T	692	0.0207	0.1091	0.8495
1	rs4373767	*ZC3H11B*	217826305	T	692	0.0118	0.09535	0.9016
17	rs2969180	*SHISA6*	11348626	A	666	-0.01123	0.09743	0.9083
10	rs10882165	*CYP26A1*	94914314	T	665	0.003704	0.09707	0.9696

Table 5.2 The GWAS association results in TEDS (n=698) for known genetic loci for spherical equivalent from adult GWAS, ordered by significance level. The two SNPs replicated at the p<0.05 threshold are highlighted in red. Abbreviations: SNP = single nucleotide polymorphism, bp = base position, A1 = reference allele, n = no. of individuals, BETA = beta coefficient, SE = standard error, P = p value

5.224 Discussion

There were no genome-wide significant loci identified in TEDS. This is not surprising given the small sample size (n~700). As illustrated in Figure 5.6, with the numbers available and variance in spherical equivalent, I did not expect to have sufficient power to detect common genetic variants (MAF>0.1) with typical effect sizes (~0.1 - 0.2 D), and certainly insufficient power to detect rare variants with MAF<0.1 and small effect sizes of <0.1. To test the robustness of my results I also examined closely the association results for the gene *GJD2*; this loci has a high frequency in European adults and it would be expected to be significant in this sample of European children. However there was a poor degree of association (p=0.655). Only two loci from adult GWAS were replicated from adult GWAS (*A2BP1* and *PCC*); this finding is again more representative of the limited power of this analysis and no implications of how well loci from adult GWAS are replicated in childhood onset myopia can be made. In conclusion, this part of my PhD does not contribute significantly to the literature on myopia but it was important for me to acquire skills in independently performing a GWAS.

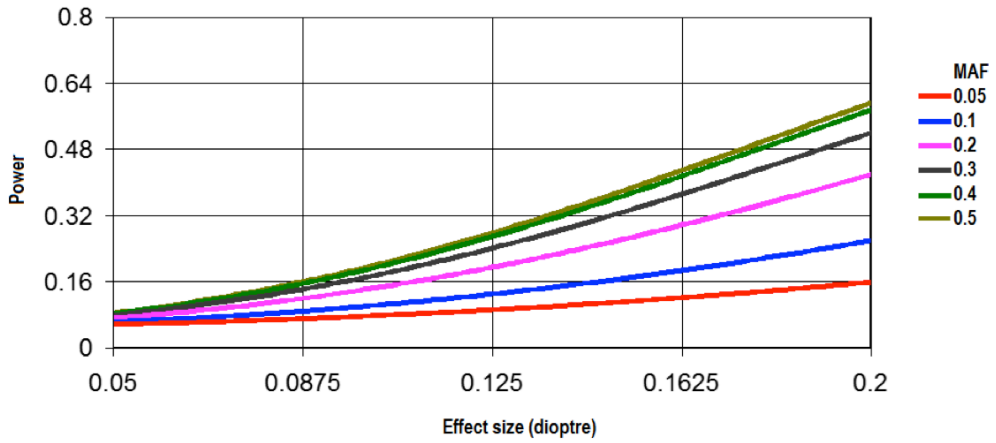


Figure 5.6 Results of power calculation for GWAS in TEDS given different effect sizes and minor allele frequencies (MAF)

5.3 The use of proxies in GWAS studies of refractive error

An alternative method to increase power in genetic association studies may be the use of proxies for spherical equivalent; a method successfully adopted by the genomics company 23andme (266). The authors of this study used the reported age of spectacle wear (AOSW) as a proxy for severity in those with reported myopia. This method has good reasoning, as it is well understood that the younger an individual wears glasses,

the more likely they are to have high myopia. I assessed this proxy in the TwinsUK adult twin registry; individuals were asked on two occasions at what age they first wore glasses and responses were consistent with 96% producing an answer with ≤ 5 years difference. This replicates previous research suggesting the age that one first wears glasses is a highly memorable event (267). The correlation coefficient between SE and AOSW in myopes was 0.47 ($p<0.000$), equating to AOSW explaining ~ 22% of the variance of SE. I moved onto to explore the strength of association with this proxy and the identified genetic traits for myopia in the TwinsUK cohort, and compare the significance levels with carefully measured spherical equivalent data, by performing sequential linear regressions using Merlin to take into account family structure. The findings, presented at the 2013 Association for Research in Vision and Ophthalmology Meeting, are illustrated below; for the majority of polymorphisms the most significant association was seen for SE. However for the genes LAMA2 and RDHS a more significant association was seen for AOSW; this confirms that AOSW may be a valid proxy and raises the question do these genes represent a more significant association with earlier onset myopia.

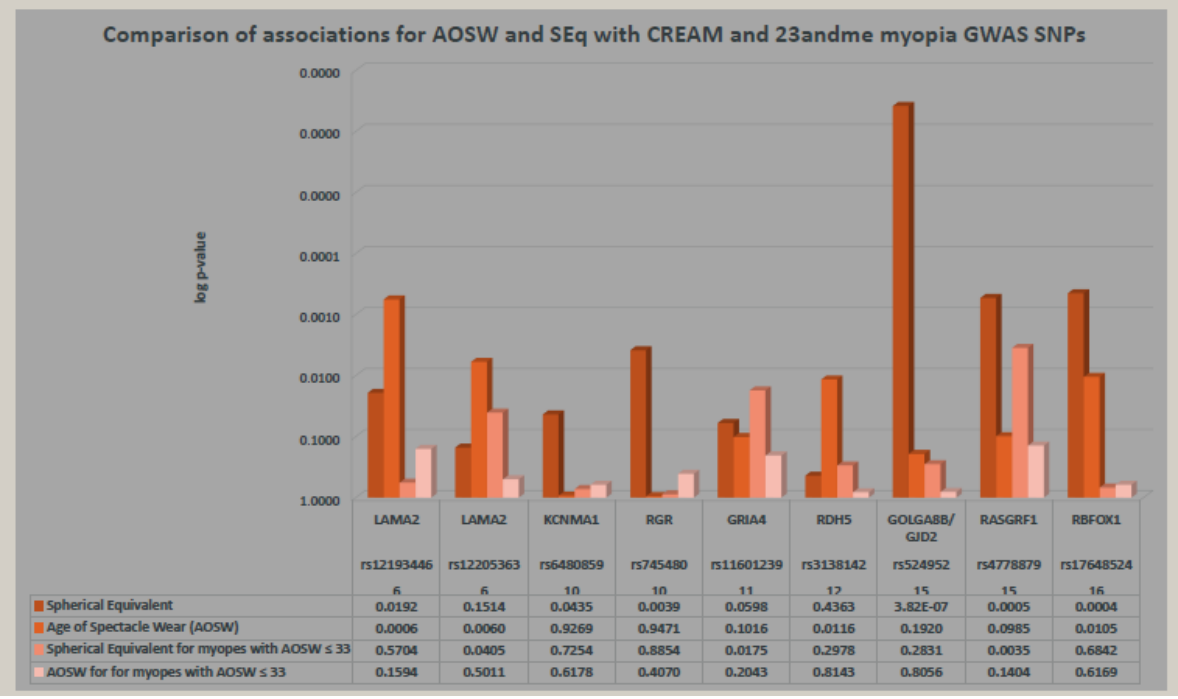


Figure 5.7 Genetic association significance levels for spherical equivalent and age of spectacle wear with previously identified SNPs in myopia

Age of spectacle wear was asked as part of the TEDS twin questionnaire and was answered by 1109 individuals. The correlation between AOSW and SE in the myopes is

very similar (r=0.43, p<0.000), illustrated below. Despite the small numbers involved (n=438), TEDS data replicates the observation that AOSW is a reasonable proxy of myopia severity.

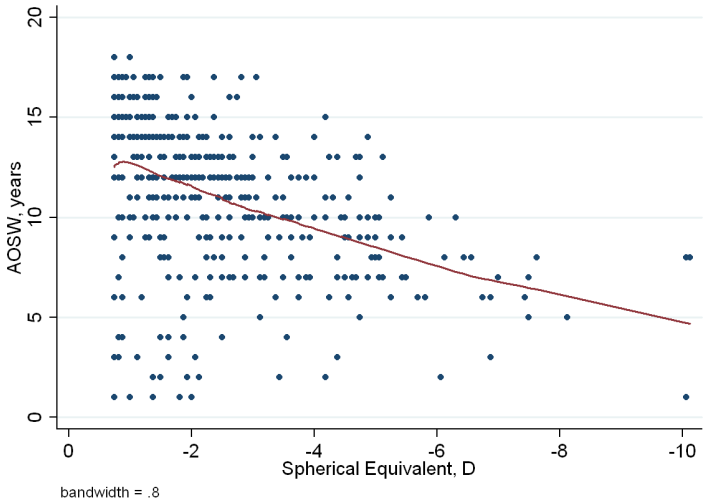


Figure 5.8 Lowess scatter plot of correlation between spherical equivalent and AOSW

An alternative proxy for spherical equivalent in the TEDS data set, are the eyesight questions from a health and wellbeing questionnaire at age 16. A total of 5576 twins were asked if they wore glasses and if they have difficulty seeing things in the distance without correction, the prime symptom of myopia, on a scale of 1-5. The correlation between spherical equivalent and the response to poor distance vision, for both all individuals (r=-0.51, p<0.01) and those who reported wearing glasses (r=-0.49, p<0.01), was high.

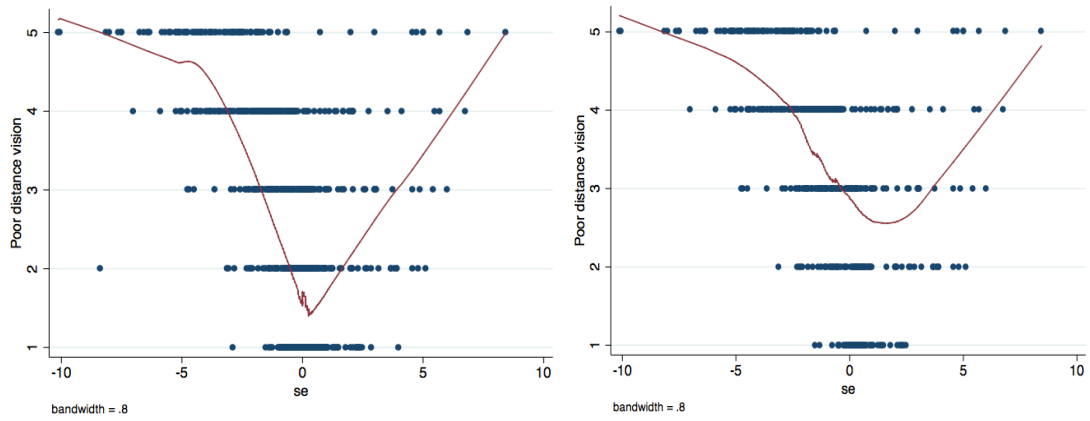


Figure 5.9 Correlation between spherical equivalent (SE) and reported scale of poor distance vision for all individuals (n=1199) and for those who reported glasses wear (n=515)

Poor distance vision rating: 1=Not difficult at all, 5=Extremely difficult

I applied this proxy (poor distance vision in all individuals) to the examination of the association between myopia and general cognitive ability (g), and compared the strength of association and significance levels to that achieved with spherical equivalent data. As shown below, spherical equivalent provides a significantly higher strength of association with g, although poor distance vision becomes a better proxy if just those who reported wearing glasses are considered.

	Poor distance vision (n=2734)		**Spherical Equivalent (n=1104)**		**Poor distance vision & glasses wear (n=869)**		**Spherical Equivalent & glasses wear (n=1104)**	
	β	**p**	β	**p**	β	**p**	β	**p**
g	0.07 (-0.01, 0.15)	0.09	-0.23 (-0.35, -0.11)	<0.01	0.31 (0.19, 0.42)	<0.01	-0.41 (-0.62, -0.20)	<0.01

Table 5.3 Comparison of the association between cognitive score 'g' and the eyesight questionnaire and spherical equivalent, adjusted for family structure, age, sex & ethnicity. β = beta coefficient of association with 95% confidence interval

5.4 Phenotypic and genotypic correlation between myopia and intelligence

This chapter is presented as a submitted paper and is an exact copy of the following journal publication:

Katie M Williams, Pirro G Hysi, Ekaterina Yonova-Doing, Omar A Mahroo, Harold Snieder, Christopher J Hammond. Phenotypic and genotypic correlation between myopia and intelligence. Scientific Reports. 2017 Apr 6;7:45977

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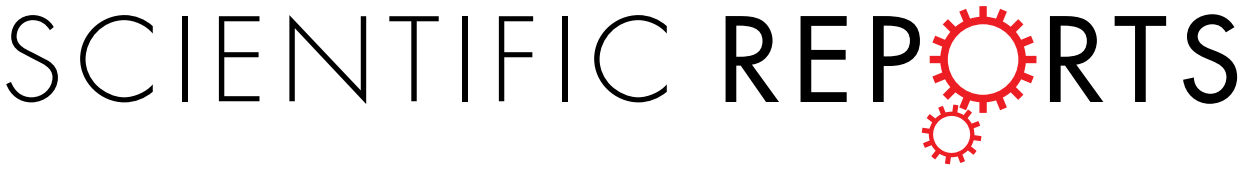

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Phenotypic and genotypic correlation between myopia and intelligence

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Myopia, or near-sightedness, is our most common eye condition and the prevalence is increasing globally. Visual impairment will occur if uncorrected, whilst high myopia causes sight-threatening complications. Myopia is associated with higher intelligence. As both are heritable, we set out to examine whether there is a genetic correlation between myopia and intelligence in over 1,500 subjects (aged 14–18 years) from a twin birth cohort. The phenotypic correlation between refractive error and intelligence was −0.116 ($p < 0.01$) - the inverse correlation due to the fact that myopia is a negative refractive error. Bivariate twin modeling confirmed both traits were heritable (refractive error 85%, intelligence 47%) and the genetic correlation was −0.143 (95% CI −0.013 to −0.273). Of the small phenotypic correlation the majority (78%) was explained by genetic factors. Polygenic risk scores were constructed based on common genetic variants identified in previous genome-wide association studies of refractive error and intelligence. Genetic variants for intelligence and refractive error explain some of the reciprocal variance, suggesting genetic pleiotropy; in the best-fit model the polygenic score for intelligence explained 0.99% ($p = 0.008$) of refractive error variance. These novel findings indicate shared genetic factors contribute significantly to the covariance between myopia and intelligence.

Myopia, or near-sightedness, occurs when a viewed object is focused in front of the retina resulting in the observer seeing a blurred image. This generally occurs a result of axial elongation, or lengthening, of the eyeball during childhood. Refractive correction in the form of glasses, contact lenses or refractive surgery is required in order for a clear image to be obtained. It is the most common ocular condition and the prevalence is increasing globally, most dramatically in urban East Asia[1–4].

Environmental factors are known to play a key role in myopia risk. There are well-established links between myopia and urbanization, lack of time outdoors, reduced light exposure, socio-economic status, prenatal factors, near work, and educational attainment[5–12]. The latter is particularly well replicated in populations around the world[3,13–15] and may reflect a number of predisposing factors; less time outdoors whilst studying, more time on near work activities, or higher intelligence. Associations between higher intelligence quotients (IQ) and myopia have been reported since Cohn's first observation in 1883[16], and subsequently in various studies internationally[15,17–24]. The relationship appears consistent in children and adults, across a range of IQ tests, and is independent of years of education completed[15,25]. Recent prospective, pediatric studies have reported up to twice the risk of myopia for those in the highest IQ quartile[18,26].

However, the nature of the association between myopia and IQ remains poorly understood. While it may reflect inadequate adjustment for reading/time outdoors, one potential explanation is that myopic children, with their cumbersome glasses, may be less likely to play sports outdoors and more likely to spend time on their school studies[27], thus attaining their full 'potential' in educational and IQ tests. Conversely, more intelligent children may spend more time reading and studying with less time in the protective outdoor environment, thereby increasing their chance of developing myopia. An alternative, but not mutually exclusive, hypothesis is that myopia and IQ may share a pleiotropic relationship[28,29]. Pleiotropy implies that a single gene may influence one or many

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	Mean age at refraction measurement (years), SD	Mean age at IQ measurement (years), SD	Sex, % male	Ethnicity, % White European	Academic achievement (mean total GCSE score), SD	Maternal education (mean highest education level), SD	Mean time spent reading (hours), SD	Mean refractive error (D), SD	Mean IQ (g), SD
MZ twins (n = 564)	16.5, 1.80	15.9, 1.04	36.6	94.9	90.5, 23.1	4.17, 1.96	5.09, 6.80	−0.61, 1.79	0.00, 0.95
DZ twins (n = 965)	16.4, 1.73	15.7, 1.27	41.2	96.2	90.2, 24.3	4.50, 2.13	4.55, 6.01	−0.30, 1.77	0.12, 1.05
Combined (n = 1529)	16.4, 1.76	15.8, 1.20	39.5	95.7	90.3, 23.9	4.37, 2.07	4.75, 6.32	−0.41, 1.78	0.07, 1.01
Participants with myopia (n = 409)	17.0, 0.82	15.9, 1.12	39.6	93.8	93.0, 24.2	4.64, 2.04	5.10, 7.50	−2.42, 1.57	0.24, 1.02
Participants without myopia (n = 1120)	16.1, 1.92	15.7, 1.25	42.8	96.5	87.9, 23.8	4.16, 2.05	4.37, 5.63	0.37, 1.07	0.02, 1.00
p value	<0.01	0.03	0.21	<0.01	<0.01	<0.01	0.08	<0.01	<0.01
Participants with genotype data	16.2, 1.79	15.8, 1.20	45.4	100	85.6, 24.1	4.00, 1.98	5.93, 8.94	−0.38, 1.70	0.01, 0.98

Table 1. Twin participant characteristics by zygosity, myopic status and genotype availability. Abbreviations: Standard deviation (SD), General Certificate of Secondary Education (GCSE), Diopters (D). Maternal educational levels graded 1–7 (none, primary school, secondary school, vocational certificate/diploma, undergraduate, postgraduate). IQ (Intelligent Quotient) score provided by g (general cognitive ability), which is a composite score of tests of verbal and non-verbal cognitive ability.

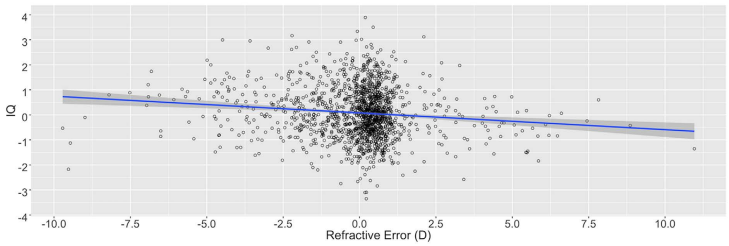

Figure 1. Scatter plot of refrective error against IQ (both variables adjusted for age and sex) with linear regression line and 95% confidence region [n = 1529].

apparently unrelated phenotypes, but to date this theory has not been tested in respect to myopia and IQ. Shared genetic factors pre-determining the risk for both traits is an interesting hypothesis made more plausible by the fact that both traits are significantly heritable; refractive error is 70–80% heritable[30,31], whilst IQ is 30–60% heritable, increasing with age[32]. Recent genome-wide association studies (GWAS) have identified genetic variants associated with refractive error and with childhood IQ[33,34].

The twin model provides the 'perfect' natural experiment to examine the relative effect of genes and environment on trait variation. The technique allows global estimation of genetic and environmental effects regardless of underlying genes or specific environmental factors[35]. Previous twin research has suggested shared genetic factors between refractive error and axial length, and, albeit with limited power, myopia and educational attainment[36,37]. In this study we explore to what extent the genetic risk between IQ and myopia is shared, utilizing a longitudinal twin birth cohort, the Twins Early Development Study (TEDS). Additionally, using genome-wide genotyping data, we created polygenic risk scores from genome-wide association studies of myopia and of IQ to predict the variance of the alternate trait.

Results

Data on refractive error and IQ were available for 1529 twin subjects, summarized in Table 1. The prevalence of myopia (≤−0.75 Dioptres (D)) in the sample was 26.7%. Individuals with myopia were significantly older (17.0 vs 16.1 years, $p < 0.01$) and had a greater proportion of non-white ethnicity (93.8% vs 96.5% white ethnicity, $p < 0.01$). The academic achievement (93.0 vs 87.9 mean total General Certificate of Secondary Education score, $p < 0.01$), IQ scores (0.24 vs 0.02 general cognitive ability 'g' score, $p < 0.01$), and maternal educational levels (4.64 vs 4.16 mean highest educational level, $p < 0.01$) were all significantly higher in those with myopia.

The phenotypic correlation between refractive error and IQ, adjusted for the effects of age and sex, was −0.116 ($p < 0.01$) [Fig. 1] - the negative correlation is due to the fact that myopia constitutes a negative refractive error. In a univariant linear model IQ was significantly associated with refractive error (beta coefficient (β) −0.217 $p < 0.01$, adjusted for relatedness only) and explained 1.5% of the variance. In a multiple linear regression

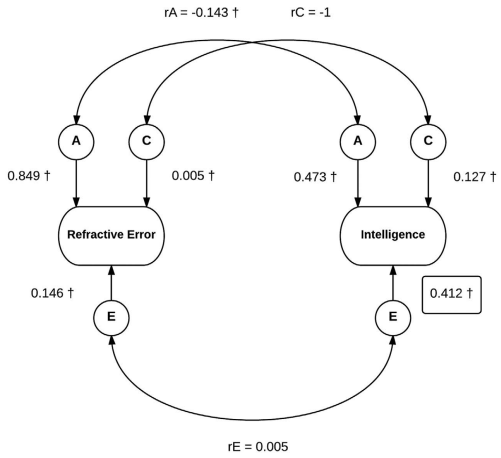

Figure 2. Bivariate twin ACE model for refractive error and IQ. Path estimates with 95% confidence intervals; A = additive genetic factors, C = common environmental factors, E = unique environmental factors; †=significant path estimates [n = 1529].

model incorporating known associations with myopia (namely age, sex, ethnicity, maternal education, academic achievement and time spent reading books) IQ remained a significant predictor (β −0.145, p = 0.02). Logistic models (adjusted for significant associations in univariant analyses) revealed an odds ratio (OR) for myopia of 1.18 with increasing IQ scores ($p < 0.01$, 95% Confidence Interval (CI) 1.02–1.37), and for those in the highest IQ quartile the risk of myopia was one and half times greater compared to those in the lowest quartile (OR 1.56, p = 0.02, 95% CI 1.08–2.25).

Twin modeling. Bivariate twin modeling estimated the heritability of refractive error at 85% (95% CI 79.9–87.5) and at 47% (95% CI 36.7–57.8) for IQ [Fig. 2]. Shared environmental factors contributed significantly to IQ variance (13%, 95% CI 3.9–21.2) and a lesser extent to refractive error (0.5%, 95% CI < 0.01–5.0). Individual environmental factors accounted for 15% (95% CI 12.3–17.4) of refractive error variance and a greater proportion of IQ variance (40%, 95% CI 36.5–43.9).

The genetic correlation (*rA*), that is the correlation between the genetic influences on refractive error and the genetic influences on intelligence, was −0.143 (95% CI −0.013 to −0.273). Shared genetic effects can be estimated as follows: $\sqrt{A}(\textit{Refractive Error})\ x\ rA\ x\ \sqrt{A}(IQ)$. This gives an estimate of 0.091. Therefore, it can be calculated that the proportion of phenotypic correlation between refractive error and IQ due to shared genetic effects is 78%.

Polygenic Risk Scores. Polygenic risk scores (PRS) can be used to estimate the degree of phenotypic variance explained by the contribution of thousands of common genetic variants (SNPs) previously associated with the trait of interest or, in this analysis, an alternate trait. PRS for childhood IQ were calculated for all unrelated individuals in TEDS with genome-wide association data using eight thresholds of significance for inclusion of variants (see Methods) from the results of a GWAS for child IQ, excluding TEDS[33]. In TEDS the IQ PRS explained 1–3% of the variance of IQ (results not shown). In all PRS models, a higher IQ PRS was associated with lower refractive error (i.e. myopia). Refractive error variance predicted by the differing PRS threshold models in 696 individuals are illustrated in Fig. 3(A); refractive error variance explained was approximately 0.5–1% across all models. In the best-fitting model, a PRS (consisting of 19,318 SNPs associated with IQ at the <0.1 p-value threshold) explained 0.99% of the variance (uncorrected p = 0.008). For comparison 3.4% of refractive error variance was explained by SNPs directly associated with refractive error in a GWAS of adults[34].

Similarly, a polygenic risk score for refractive error was calculated on all genotyped participants using eight thresholds of significance from the results of a GWAS for refractive error in adults[34]. In TEDS the refractive error PRS explained 1–2.5% of refractive error variation (results not shown), comparative to the aforementioned figure of 3.4% variance explained in adults[34]. Again, a lower refractive error PRS (ie. myopia) was associated with a higher IQ. The results of the different PRS threshold models in 1517 individuals are illustrated in Fig. 3(B); IQ variance explained was approximately 0.1–0.4% across the models. In the best-fitting model, 178 refractive error-associated SNPs explained 0.44% of IQ variance (uncorrected p = 0.01).

Discussion

Twin participants with high IQ (highest quartile) were one and a half times more likely to be myopic, comparable to others[18,26]. IQ alone explained 1.5% of refractive error variance and remained a significant predictor when adjusted for educational attainment, contrary to others[38]. In twin modeling both traits were heritable (refractive error 85%, IQ 47%), and genetic factors explained the majority (78%) of the phenotypic correlation (r = 0.12) between IQ and refractive error. Reciprocal genome-wide PRS significantly predicted the variance of

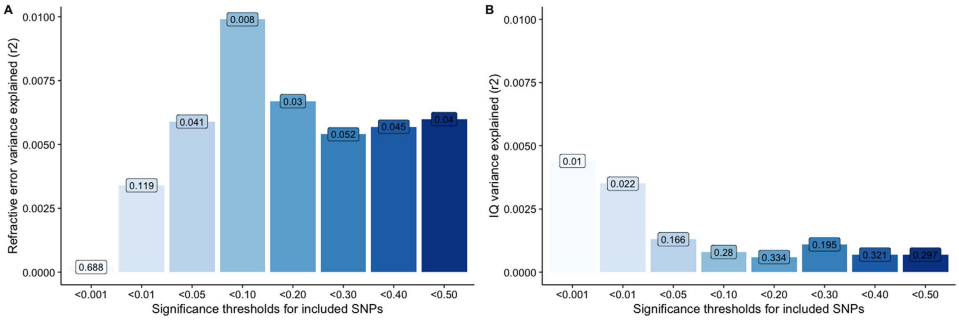

Figure 3. Genome-wide polygenic scores for intelligence (**A**) and refractive error (**B**) respectively predict variance in refractive error and intelligence. Polygenic risk scores were created using different significance thresholds for inclusion of SNPs (<0.001 to <0.50). The uncorrected p-values above each bar indicate the statistical significance of the association between the polygenic score and the respective trait. (**A**) n = 696, adjusted for two principal components, age and sex. (**B**) n = 1517, adjusted for two principal components, age and sex.

both refractive error (~1%) and IQ (~0.4%). These analyses provide evidence for genetic correlation between myopia and IQ, with shared genetic factors underlying a small proportion of variance in both traits.

To our knowledge no previous study has examined the extent of shared genetic factors for myopia and IQ. Previous bivariate twin analyses for myopia have examined the relationship with axial length and with educational attainment[36,37]. Dirani *et al.* reported that 76% of the phenotypic sharing between refractive error and educational attainment was due to shared genetic factors[36], similar to the findings in this study, however their genetic correlation was not statistically significant. Genome-wide PRS for IQ and refractive error significantly predict variation in refractive error and IQ respectively. The reciprocal PRS identified genetic effects on both traits, thus indicating a degree of genetic pleiotropy without the implication of any causal direction of effect. The degree of trait variance explained was greatest for the IQ PRS and refractive error (0.99%). Interestingly only 3.4% of refractive error variance in adults is explained by genetic variants specifically associated with the refractive error in previous GWAS[34], and in our analysis this figure was 1–2.5%, which is not incomparable to the variance explained by 'IQ SNPs'.

The argument that highly intelligent children, who may spend more time on near-work activities, increase their risk of developing myopia has long been favored[8,9,39]. However the association between near-work and myopia is inconsistent[40,41], and importantly refraction in young children, prior to the experience of intensive education and near-work, can significantly predict those who will later become myopic[42,43]. The possibility that increased time studying can increase an individual's IQ has been largely discounted; there is no robust evidence that a large effect on IQ can be achieved by such an intervention[44]. Shared genetic factors may play a role in both traits. Early proponents for this theory identified an association between myopia and IQ where the intellectual gain preceded the development of myopia, and differential status in siblings[28,45,46]. A single myopia gene that influenced brain development with evolutionary advantages for urbanized living was proposed[28,29,47]. The idea of a single gene controlling brain development and eye growth, in light of modern knowledge of the polygenicity of both traits, now appears implausible. However, the possibility of a number of genes of small effect, perhaps inherited simultaneously and linked, that control neural signaling influencing ocular growth and learning abilities remains interesting. Recent research has identified gene-environment interactions between educational attainment and myopia[48], whilst PRS for educational attainment predict refractive error and when incorporated as an instrumental variable in Mendelian randomization (MR) analysis support the notion that educational attainment is causally related to refractive error[49]. The education PRS in the MR study only explained 0.25% of refractive error variance, comparatively less than the variance explained by IQ in our analysis. We would argue that IQ may be a 'purer' phenotype for analysis than educational attainment, which incorporates many underlying factors.

In axial myopia, the commonest form of myopia, elongation of the eye results in a focused image falling in front of the retinal plane. The retina, together with a number of other ocular structures, including the ciliary body which controls accommodation (near focus), originate embryonically from the same tissue as the brain (neuroectoderm)[50]. Retinal signaling, through detection of focused light, influences scleral remodeling and ultimately ocular axial length[51,52]. Therefore, could the same genetic factors in the retina and brain theoretically be involved in the regulation of both structures? There is some evidence that people with high IQ have a 'larger brain', with correlations estimated at 0.38 to 0.45[53,54]. Brain size itself does not predict cognitive ability within families[55], although incorporating other neuroimaging variables can provide a modest prediction of IQ variance[56].

Globally, myopia is becoming more common and IQ scores are rising[1,57]. Some have argued that an evolutionary drive may have increased the human population frequency of pleiotropic genes for higher IQ and myopia[58]. The main limitation of this theory is the temporal relationship; evolutionary changes occur over multiple generations whereas the increases in myopia and IQ scores have been observed within the last century. It is likely for myopia that aspects of modern day childhood are more attributable, but it is interesting to note the heritability of IQ increases over childhood and across the lifespan - this has been attributed to genetic amplification, most

likely through gene-environment effects rather than additional genetic influences, as the same genes appear to be involved in cognitive ability at different ages[59,60].

This analysis utilizes a powerful twin data set with phenotypic and molecular genetic data; for the IQ PRS comprising of SNPs over a significance threshold of 0.1, we have enough statistical power to detect a genetic covariance of 0.09 between IQ and refractive error[61]. Although the gold standard of cycloplegic autorefraction was not used, at age 14–18 the subjects were old enough for subjective refraction, using techniques to avoid excessive diagnosis of myopia such as use of the duochrome bar. In large epidemiological studies of adults this method introduces minimal bias[62], whilst in younger populations it has been found that whilst there is a large degree of inaccuracy in children <10 years, in older teenagers the degree of potential inaccuracy is less[63]. The association with IQ was examined using a composite variable (g); this composite score was comprised of verbal and non-verbal tests created to reliably measure general cognitive ability at each age. The technique of twin modeling provides an upper limit estimate of heritability and therefore genetic relationships, whilst it tends to underestimate the effect of shared environmental factors[31]. Although an ADE twin model was marginally better-fitting than the ACE model for refractive error (providing the same heritability estimate of 86%), we elected to use matched ACE models for both traits as this enabled the examination of any potential differences in shared and unique environmental effects. Polygenic risk scores are limited to testing the effect of common genetic variation and the additive genetic model of inheritance, unlike twin modeling. This means the captured genetic contribution is an underestimate of the genetic contribution to trait variance, which may additionally include rare variants, gene-environment interactions and epigenetic effects. Polygenic risk scores are designed to test whether SNPs that do not reach genome-wide significance in a discovery GWAS explain a significant proportion of variation in a trait in an independent sample. The fairly liberal thresholds used will mean many non-associated SNPs are in the score and for this reason the term 'polygenic risk score' rather than 'genetic risk' is used. The premise is that collectively these SNPs account for a substantial proportion of variation. Despite appropriate measures for adjusting for ancestry, genetic heterogeneity between TEDS and the discovery GWAS results used may influence the degree of association in polygenic risk scores, although we suspect observed differences in association are more likely to be due to the differing success of the discovery GWAS. Polygenic risk scores were also subject to limited power as the sample of genotyped twins was relatively small. The p values have not been corrected for the issue of multiple testing which is a limitation. GWAS for intelligence on larger sample sizes have been performed[64,65]; however, these were conducted on adult participants and explain a smaller amount of IQ variance in TEDS compared to that explained by the childhood IQ GWAS used for this study[33]. Finally, no directionality or causality can be inferred from these methods, and the complex effect of gene-environment interaction is not incorporated.

In summary, our bivariate twin model suggests that shared genetic factors underlie the majority of the phenotypic correlation between myopia and IQ. This was substantiated using molecular genetic data where approximately 1% of refractive error variance was explained by genetic variants linked to IQ. This provides novel evidence for a modest but significant contribution of pleiotropic genetic factors contributing to the development of myopia and higher intelligence.

Materials and Methods

Participants. The Twins Early Development study (TEDS) is a longitudinal birth cohort of twins studied from a neurodevelopmental perspective using multivariate quantitative and molecular genetic techniques. In the initial TEDS study over 15,000 families of twins born in England and Wales in 1994, 1995 and 1996 were recruited. The sample remains representative of the UK population[66]. Ethical approval for all experimental protocols TEDS and the TEDS myopia study has been provided by the Institute of Psychiatry ethics committee. All methods were carried out in accordance with relevant guidelines and regulations. A subset of 2625 families was selected for the TEDS Myopia study. This sample was selected to include families from TEDS where twins had completed a questionnaire that included eyesight questions, and additional families where twins had genotype data. We excluded from the analyses children with severe current medical problems and families who were not contactable or who lived overseas.

Measures. Postal questionnaires were sent to 2625 families inviting participation in the myopia study and consent was requested from the parents, as well from the twins, to contact their optician for a recent refraction. A response rate of 51.7% of potential twin participants was achieved (n = 2715). Responders and non-responders were comparable in terms of ethnicity, gender, zygosity, and parental employment; however there was a slightly higher rate of twin and parental secondary school examinations achievement in the responders. Study questionnaires were sent to the optometrists given by 2,283 twins, from whom informed consent was obtained, requesting a brief ophthalmic and refractive history together with a most recent refraction. Non-cycloplegic subjective refractive error measurements were obtained for 1996 individuals (majority 70% aged 16–18, 92% aged 14–18 at their most recent refraction). Spherical equivalent (SE) was calculated using the standard formula (SE = sphere + (cylinder/2)) and the mean of the two eyes was considered for each individual. Myopia was defined as SE ≤ -0.75 diopters (D). Standardized residuals of mean spherical equivalent adjusted for age and sex were calculated (n = 1991).

Multiple child and parent questionnaires, in addition to teacher questionnaires, web-based testing and home assessments, have been conducted over the twins' life-course. General cognitive ability or g[67] was assessed using a combination of parent-administered, phone- and web-based tests at ages 2, 3, 4, 7, 9, 10, 12, 14 and 16 years of age. At each age the twins completed at least two ability tests that enabled assessment of verbal and non-verbal intelligence. For this study the measurement of g factor, which is essentially a measure of IQ, was taken from the oldest ages of testing, as this was the age in closest approximation to the age of refraction. At these ages subjects completed a web-based adaptation of Raven's Standard and Advanced Matrices, and the Mill-Hill Vocabulary

Scale[68–70]. Age and sex adjusted standardized residuals at age 16, imputed with age 14 if missing, were calculated for 1529 individuals. Time spent reading was asked on a child questionnaire at the age of 14, where enjoyment and hours spent per week on various hobbies and activities was assessed.

Genotyping. Genotyping was performed on 3665 individuals (one twin per pair) on Affymetrix GeneChip 6.0 single nucleotide polymorphisms (SNP) genotyping arrays (Affymetrix, Santa Clara, CA, USA) using standard experimental protocols, as part of the WTCC2 project. Twins excluded from genotyping were children with serious medical or perinatal problems, non-white ethnic origin, and English spoken as a second language at home." A total of 3152 DNA samples (1446 males and 1706 females) survived quality control for relatedness, hetereozygosity, ancestry and hybridization intensity outliers. Genotypes at untyped markers were imputed with HapMap phase II and III, and WTCCC2 controls using IMPUTE (v2). The quality control criteria used to select imputed SNPs were an information score of $\geq$0.90 for WTCCC2 controls and $\geq$0.98 for HapMap imputation. Zygosity was assigned using parental questionnaires of physical similarity; this has shown over 95% accuracy when compared to DNA testing[71]. DNA testing was performed when zygosity was unclear.

Statistical Analysis. Correlation coefficients between refractive error and IQ, adjusted for age and sex, were calculated. The association and variance explained (r^2 or coefficient of determination) for refractive error with IQ was observed in univariate and multiple linear regression models, adjusting for some of the known risk factors for myopia with refractive error considered as a continuous trait and myopia as a binary trait (≤ -0.75 D). The association between different quartiles of IQ and also non-verbal and verbal IQ were evaluated. In analyses $p < 0.05$ was considered statistically significant.

Bivariate twin Modeling. In twin modeling the phenotypic variance of a trait is partitioned into three factors: additive genetic effects (A), non-additive genetic effects (D) or the shared environment between siblings (C), and the individual-specific environment effects (E). Monozygotic pairs (MZ) have the same genetic and shared environmental effects, whereas in dizygotic twins (DZ) additive genetic effects are 50% correlated, but the shared environmental effects are the same. An ACE model, rather than an ADE model, was chosen based on the twin correlations for the traits and in an effort to examine any potential differences in shared and unique environmental effects on the traits. We performed standard ACE model-fitting analysis using the OpenMx package (http://openmx.psyc.virginia.edu) in R (http://www.R-project.org). Heritability (or h^2) is provided by the estimate for additive genetic effects. A bivariate Cholesky decomposition model for IQ and refractive error was constructed to assess the phenotypic variance and covariance attributable to genetic and environmental factors. There are two mathematically equivalent solutions to the bivariate model and for this analysis we selected the correlated factors solution which does not assume that the factors underlying the first variable influence the second variable and allows ascertainment of the proportion of phenotypic correlation due to A, C & E. Age and sex adjusted standardized residuals were used for both traits. The degree of phenotypic correlation explained by genetic factors was calculated by dividing the estimate of the shared genetic effects by the phenotypic correlation.

Polygenic Risk Scores. Polygenic risk scores (PRS) enable estimation en masse of the genetic contribution of thousands of common variants, generally of small individual effects, on the variation of a trait. In this study, estimation, at a participant level, of the degree of variance of one trait (eg refractive error) explained by the SNPs associated with a second trait (eg. IQ) from an unrelated sample was examined. The results from a large international meta-analysis GWAS for IQ[33] in children of European ancestry were used to calculate an IQ PRS (results selected that did not include TEDS participants), whilst a similar GWAS for refractive error in adults (limited to those of European ancestry) was used to calculate a refractive error PRS[34]. From the GWAS results the SNP, reference allele, beta coefficient and p value were extracted. For each individual the quality controlled SNPs were pruned for linkage disequilibrium using a clumping approach in Plink v1.9 using a pairwise cut-off of $r^2 \leq 0.25$ within 200 kB window, and a MAF cut-off of $>$0.03. This resulted in 120481 SNPs. Individualized PRS, for both refractive error and IQ, were calculated with eight thresholds for inclusion of trait-associated SNPs, so that the effect of a range of SNPs on the alternate trait could be examined. The eight significance thresholds tested were: p value < 0.001, <0.01, <0.05, <0.1, <0.2, <0.3, <0.4 and <0.5. General linear models were constructed for IQ-associated SNPs and refractive error with age, sex and the first two ancestry-informative principal components were included as covariates in 696 unrelated individuals (one twin per pair), and then vice versa in 1517 individuals. In each model the significance of the PRS (assessed by an uncorrected p-value, with <0.05 considered significant) and variance explained (assessed by r2) was observed. Analysis was performed using Plink v1.9[72] and Stata version 13.1 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

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Author Contributions

K.W. designed and performed the research, performed data analysis, and wrote the paper; E.Y. & O.M. assisted with data analysis and writing the paper; P.H. & H.S. assisted with research design, data analysis and paper preparation; C.H. assisted with research design, data analysis and writing the paper.

Additional Information

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5.5 Childhood gene-environment interactions

This chapter is presented as a published paper and is an exact copy of the following journal publication. Gene by environment analyses require large numbers of participants to obtain significant results – as such I would have no useful results by running these types on analysis on my data alone and therefore joined collaborative efforts to increase power (as part of the Consortium for Refractive Error and Myopia (CREAM)). As fourth author on this paper I ran the analyses on the Twins Early Development Study and contributed my findings. I was also involved with reviewing and amending the final manuscript in the pre-submission and publication stages.

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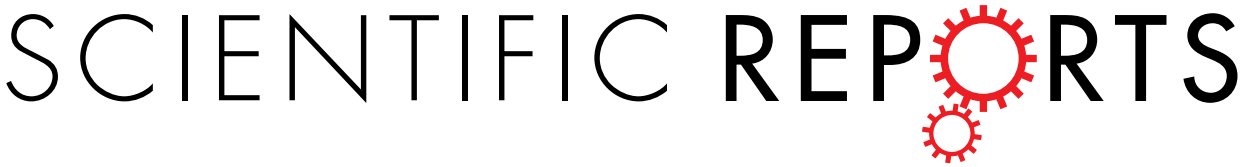

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Childhood gene-environment interactions and age-dependent effects of genetic variants associated with refractive error and myopia: The CREAM Consortium

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Myopia, currently at epidemic levels in East Asia, is a leading cause of untreatable visual impairment. Genome-wide association studies (GWAS) in adults have identified 39 loci associated with refractive error and myopia. Here, the age-of-onset of association between genetic variants at these 39 loci and refractive error was investigated in 5200 children assessed longitudinally across ages 7–15 years, along with gene-environment interactions involving the major environmental risk-factors, nearwork and time outdoors. Specific variants could be categorized as showing evidence of: (a) early-onset effects remaining stable through childhood, (b) early-onset effects that progressed further with increasing age, or (c) onset later in childhood (N = 10, 5 and 11 variants, respectively). A genetic risk score (GRS) for all 39 variants explained 0.6% (P = 6.6E–08) and 2.3% (P = 6.9E–21) of the variance in refractive error at ages 7 and 15, respectively, supporting increased effects from these genetic variants at older ages. Replication in multi-ancestry samples (combined N = 5599) yielded evidence of childhood onset for 6 of 12 variants present in both Asians and Europeans. There was no indication that variant or GRS effects altered depending on time outdoors, however 5 variants showed nominal evidence of interactions with nearwork (top variant, rs7829127 in *ZMAT4*; P = 6.3E–04).

The refractive errors myopia and hyperopia are common visual disorders that typically require correction with spectacles, contact lenses, or refractive eye surgery. Myopia – particularly with increasing severity – is a leading cause of irreversible visual impairment and blindness due primarily to stretching and thinning of the ocular tissues within the posterior segment of the eye. These changes are associated with an increased risk of retinal detachment, chorioretinal atrophy, choroidal neovascularisation, myopic maculopathy, glaucoma and cataract[1,2]. Myopia is rare in infancy, usually developing during school age or in early adulthood[3]. For current generations of young adults, approximately 30–40% of individuals in Western countries[4,5] and 80% of those in urban areas of East Asia[6,7] have myopia.

Genome-wide association studies (GWAS) in primarily population-based samples[8–14] and next-generation sequencing (NGS) studies of carefully selected high myopia pedigrees harbouring extremely rare, high penetrance disease-causing mutations[15–20] have improved our understanding of the genetics of refractive error and myopia. To date at least 39 distinct loci harbouring common genetic variants showing genome-wide significant association with refractive error have been identified through GWAS. For the genetic variants that contribute most to the burden of myopia in the general population (i.e. the GWAS-identified variants) it is not yet known whether the variants act during very early life, childhood, or in adulthood. This is an important question given that knowledge of the time and mode of action of the causal variants at the associated loci is necessary for detecting children at-risk of myopia (who would benefit most from treatment intervention), and would aid the design of new therapies capable of halting myopia progression.

For environmental risk factors to which most children are exposed, inter-individual differences in genetic susceptibility may account for some of the phenotypic variance[21]. Exposure to nearwork, i.e. reading and other tasks requiring prolonged near vision, has long been proposed as an environmental risk factor for myopia to which children are ubiquitously exposed during their schooling. The total duration of reading, the period of continuous reading, the reading distance between the text and the eyes, and variation in nearwork exposure outside of the school day have each been shown to be associated with refractive error or myopia progression[22,23]. The other most strongly implicated environmental risk factor for myopia is insufficient time spent outdoors[24–26], and it has been suggested that time spent outdoors and time spent performing nearwork activities together underlie the robust association between myopia and educational achievement[2,27]. Gene-environment (GxE) interactions – which in this project we define as marker-phenotype associations whose effects differ statistically depending on whether individuals have been exposed to a high vs. low level of an environmental risk factor – may contribute extensively to variation in disease susceptibility[28]. Given the recent identification of gene-environment interactions involving nearwork or level of education, a key question in myopia research currently is whether GxE interactions contribute to the rising prevalence of myopia and to the higher incidence rate observed in young Asian populations as compared to their European counterparts.

We carried out analyses of pediatric/adolescent cohorts collaborating in the Consortium for Refractive Error And Myopia (CREAM) to investigate whether the top index variants at the 39 loci previously identified in GWAS meta-analyses of adults have early-onset effects manifest during childhood. We also tested for evidence of GxE interactions involving either nearwork or time spent outdoors. A single large cohort with longitudinal measurements of refractive error over much of childhood was used for the primary analyses. Meta-analyses of cross-sectional samples were then used to test for replication.

Methods

Participants and phenotypes. All participants were aged <25 years-old and none had been included in the earlier CREAM meta-analysis of refractive error[9], which only included individuals >25 years of age. Details of the participant recruitment and phenotypic assessment are presented in the Supplementary Information. The study was conducted in accordance with the Declaration of Helsinki, and all participants provided informed consent. The experimental protocols for the study were approved by the respective ethical review boards at host institutions, as follows. ALSPAC, the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees; BATS, the Human Research Ethics Committee at the QIMR Berghofer Medical Research Institute; GZT, the Ethics Review Board of the Zhongshan Ophthalmic Center of Sun Yat-Sen University; RAINE, the Human Research Ethics Committee at the University of Western Australia; SCORM and STARS, the Institutional Review Boards of the Singapore Eye Research Institute, Singapore General Hospital, National University of Singapore, and the National Healthcare Group, Singapore; TEDS, the Institute of Psychiatry ethics committee; TEST, the

Longitudinal cohort (N = 5,200)					
Study	**Ethnicity**	**N**	**Female (%)**	**Age-at-baseline**	**Years follow-up**
ALSPAC*	European	5200	51.0	7.5 (0.3)	7.0 (1.5)
Cross-sectional cohorts (N = 5,599)					
Study	**Ethnicity**	**N**	**Female (%)**	**Age (years)**	**Refraction (D)**
TEDS	European	698	56.0	16.2 (1.8)	−0.38 (1.70)
WESDR	European	289	50.5	17.7 (4.6)	−1.09 (1.79)
TEST	European	410	57.2	11.8 (5.0)	0.36 (1.24)
RAINE	European	754	50.9	20.0 (0.4)	−0.06 (1.53)
BATS	European	992	53.6	19.1 (3.2)	−0.33 (1.42)
GZT	Asian	1055	51.8	15.6 (2.8)	−1.97 (2.49)
SCORM	Asian	994	48.4	7.5 (0.9)	−0.55 (1.73)
STARS	Asian	407	49.4	6.6 (3.9)	−2.00 (2.09)

Table 1. Demographics of study samples. Values in brackets are standard deviations. *Refraction details at each age for the longitudinal cohort are provided in the supplementary material (Table S8).

Royal Victorian Eye and Ear Hospital, the University of Tasmania, and the Australian Twin Registry; WESDR, the Health Sciences Institution Review Board of the University of Wisconsin, Madison.

Participants underwent cycloplegic autorefraction (RAINE, TEST, BATS, GZT, SCORM, STARS) or non-cycloplegic autorefraction (ALSPAC) or subjective refraction (TEDS, WESDR) and the spherical equivalent refractive error averaged between the two eyes was calculated. Parental questionnaires that included items on time spent engaged in nearwork outside of school, and time spent in outdoor activities were used to classify children as spending a high or low amount of time performing nearwork (Table S6) or outdoors (Table S7) each day. Classification was done within each cohort separately, using a median split ("low" group, exposure below median level; "high" group, exposure above median level).

Genetic analysis. DNA samples obtained from blood or saliva were genotyped using either an Illumina or Affymetrix high-density single nucleotide polymorphism (SNP) array, and genotypes at untyped markers were imputed using the 1000-Genomes Project reference panel (see Table S5 for details). Stringent quality control procedures (e.g. imputation quality r^2 or info score >0.5) were applied to each cohort separately (Supplementary Information). 39 SNPs that showed genome-wide significant association with refractive error in the general adult population in two previous GWAS analyses[8,9] were selected for evaluation (Table S1).

Cross-sectional models and meta-analyses. For each of the 8 cross-sectional cohorts separately, single SNP tests of association with refractive error were conducted using the following linear regression model:

$$y_i = m + a_i\beta_{Age} + s_i\beta_{Sex} + g_i\beta_{SNP} + \varepsilon_i \quad (1)$$

where y_i is the spherical equivalent refractive error of the i^{th} participant, of age a_i and sex s_i and with g_i their risk allele dosage on the scale 0–2 for the test SNP, and ε_i the residual. Regression coefficients are indicated as β_{Age}, β_{Sex}, and β_{SNP} for the model parameters age, sex and SNP genotype, respectively. Additional G x E interaction models were tested for samples with information available on environmental exposures, nearwork or time outdoors (both exposures coded: 0 = low, 1 = high). For the i^{th} participant, using n_i to denote nearwork and t_i for time outdoors:

$$y_i = m + a_i\beta_{Age} + s_i\beta_{sex} + g_i\beta_{SNP} + n_i\beta_{NW} + g_in_i\beta_{SNP\cdot NW} + \varepsilon_i \quad (2)$$

$$y_i = m + a_i\beta_{Age} + s_i\beta_{sex} + g_i\beta_{SNP} + t_i\beta_{TO} + g_it_i\beta_{SNP\cdot TO} + \varepsilon_i \quad (3)$$

Results from the individual cohorts were meta-analyzed in 5599 individuals comprising 5 cohorts of European ancestry (BATS, RAINE, TEDS, TEST, WESDR; N = 3,143; Table 1) and 3 cohorts of Asian ancestry (GZT, SCORM, STARS; N = 2,456; Table 1) using a weighted inverse-variance, fixed effects model[29]. A random effects model was used if Cochran's Q-test for heterogeneity yielded a P-value below 0.05.

Longitudinal study (ALSPAC). Refractive error was included in the clinical assessments for ages 7, 10, 11, 12 and 15 years in ALSPAC children[30]. Linear mixed models for refractive trajectory were fit as described[30] using the *nlme* package in R[31] for individuals (N = 5,200; Table 1) who underwent at least 3 refractive assessments and whose genotype data passed quality control filters (as described in the Supplementary Information). Briefly, SNP dosage, age and higher-order age terms (age^2 and age^3) were modelled as fixed effects while for each child, the difference from the average refractive error at baseline and the linear rate of change in refractive error were modelled as individual-level random effects, using an autoregressive correlation structure. To examine GxE interactions, initially, 3-way interaction models were tested that included the interaction between SNP, change-from-baseline in age, and environmental exposures (nearwork or time outdoors). If the P-value for the 3-way interaction was >0.05 then models including only 2-way interactions were tested.

Quanto[32] was used to gauge the power to detect main and interaction effects in the ALSPAC cohort. These calculations assumed a minor allele frequency (MAF) of 0.25, a sample size of 4461 (corresponding to 5,200 minus

					SNP main effect at baseline (D)			SNP x Age interaction (D/yr)		
Marker	Chr	Gene	RA	RAF	Beta	SE	P	Beta	SE	P
GR Score	–	–	–	–	**−0.018**	**0.003**	**2.2E–09**	**−0.003**	**0.000**	**5.8E–14**
rs1652333	1	CD55	G	0.32	−0.002	0.019	9.3E–01	**−0.005**	**0.003**	**4.0E–02**
rs1656404	2	PRSS56	A	0.21	**−0.066**	**0.024**	**5.7E–03**	**−0.008**	**0.003**	**1.3E–02**
rs1881492	2	CHRNG	T	0.23	**−0.058**	**0.024**	**1.7E–02**	−0.005	0.003	1.5E–01
rs14165	3	CACNA1D	G	0.70	**−0.040**	**0.020**	**4.2E–02**	−0.001	0.003	7.7E–01
rs7744813	6	KCNQ5	A	0.59	**−0.048**	**0.019**	**9.9E–03**	**−0.005**	**0.003**	**3.5E–02**
rs12205363	6	LAMA2	T	0.92	**−0.097**	**0.035**	**5.7E–03**	−0.008	0.005	1.2E–01
rs7837791	8	TOX	G	0.53	**−0.045**	**0.018**	**1.1E–02**	**−0.005**	**0.002**	**2.7E–02**
rs4237036	8	CHD7	T	0.66	0.020	0.019	2.9E–01	**−0.007**	**0.003**	**5.6E–03**
rs7042950	9	RORB	G	0.22	0.018	0.022	4.1E–01	**−0.009**	**0.003**	**2.5E–03**
rs6480859	10	KCNMA1	T	0.37	−0.029	0.018	1.1E–01	**−0.008**	**0.002**	**1.3E–03**
rs10882165	10	CYP26A1	T	0.40	**−0.035**	**0.018**	**4.8E–02**	0.001	0.003	7.6E–01
rs8000973	13	ZIC2	C	0.52	**−0.042**	**0.018**	**1.8E–02**	**−0.008**	**0.002**	**1.5E–03**
rs66913363	14	BMP4	G	0.51	**−0.051**	**0.018**	**5.3E–03**	0.001	0.003	7.2E–01
rs524952	15	GJD2	A	0.46	−0.018	0.018	3.3E–01	**−0.008**	**0.003**	**8.8E–04**
rs17648524	16	A2BP1	C	0.33	−0.001	0.019	9.4E–01	**−0.007**	**0.003**	**5.6E–03**
rs2969180	17	SHISA6	A	0.35	**−0.039**	**0.019**	**3.9E–02**	**−0.005**	**0.003**	**4.9E–02**

Table 2. Age-of-onset of SNP associations with refractive error in the discovery cohort (ALSPAC). Abbreviations: Chr = Chromosome. GR = Genetic risk. RA = Risk allele. RAF = Risk allele frequency. Associations were tested at baseline (age of 7.5 years-old) and over the next 7 years (SNP x Age interaction). Results for all 39 SNPs are shown in Table S2.

739 participants with missing information about time spent performing nearwork), a binary exposure affecting 39% of the cohort (equivalent to that for high vs. low nearwork exposure in ALSPAC) and a refractive error distribution with a mean of zero and a standard deviation of 1.50 D. The estimated power would be conservative given that a linear mixed model analysis will have greater power than a linear model analysis.

Genetic risk score for all 39 SNPs. A genetic risk score was computed by summing the dosage of risk alleles for all 39 SNPs. In individuals of Asian ancestry only 31 of the 39 SNPs were polymorphic (MAF > 0.05) and therefore contributed to the genetic risk score calculation. The frequency distribution of genetic risk score in each sample was normally distributed with a mean of 36 (95% C.I. 29 to 42) alleles in Europeans and 40 (95% C.I. 37 to 42) alleles in Asians. To calculate the variance in refractive error explained by the genetic risk score at a specific age for participants in the ALSPAC cohort, refractive error at age 7.5 years (or at age 15 years) was regressed on genetic risk score using a linear model. The covariates age and sex were not associated with refractive error when included in the age 7.5 or the age 15 year model, and their inclusion did not improve the fit of either model (note that being a birth cohort, the age range was narrow). Hence these covariates were omitted. The variance explained by the genetic risk score was therefore taken as the R^2 value for a model that included the genetic risk score as the only predictor variable.

Pathway analysis. The genes (Table 2) implicated in having early-onset effects (N = 10 genes) or later-onset effects (N = 11 genes) in the ALSPAC discovery sample were evaluated using PANTHER Version 10.0 (release date May 15, 2015)[33] and DAVID Version 6.7 (release date 27 Jan, 2010)[34] to identify potential functional pathways.

Results

Early-onset and later-onset effects in childhood. Nine cohorts of children/adolescents were studied (Table 1). The largest of these, ALSPAC (N = 5,200), which had longitudinal data for refractive error, was used for discovery analyses, and 8 cross-sectional cohorts were used for validation. The discovery cohort had ~80% power to detect an association for a SNP with an effect size of 0.1 D and MAF of 0.25.

Of the 39 SNPs examined, 16 showed evidence of onset in childhood (Table 2 and Table S2). Early-onset associations already manifest at 7.5 years of age were present for 10 SNPs (P = 4.8E–02 to P = 5.3E–03). Later-onset associations that emerged between the ages of 7.5 and 15 were noted for 11 SNPs (P = 4.9E–02 to 8.8E–04 for SNP x Age interaction). Five SNPs showed a main effect at baseline as well as later progressive effects. Examples of SNPs showing evidence of early-onset and later-onset effects are presented in Fig. 1 for early-onset *CHRNG* SNP rs1881492, later-onset *A2BP1* (also known as *RBFOX1)* rs17648524, and *PRSS56* rs1656404 with both effects. For all associated SNPs the "direction of effect" was the same as in the original GWAS[8,9].

The genetic risk score was very strongly associated with refractive error both at 7.5 years of age (β = −0.018 D, 95% CI −0.012 to −0.024, P = 2.2E–9) and with increasing age (β = −0.003 D/yr, 95% CI −0.002 to −0.004, P = 5.8E–14). By the age of 15 years, the model suggested that the 39 SNPs together would produce a more than 1.0 D difference in refractive error between participants carrying the lowest and highest number of risk

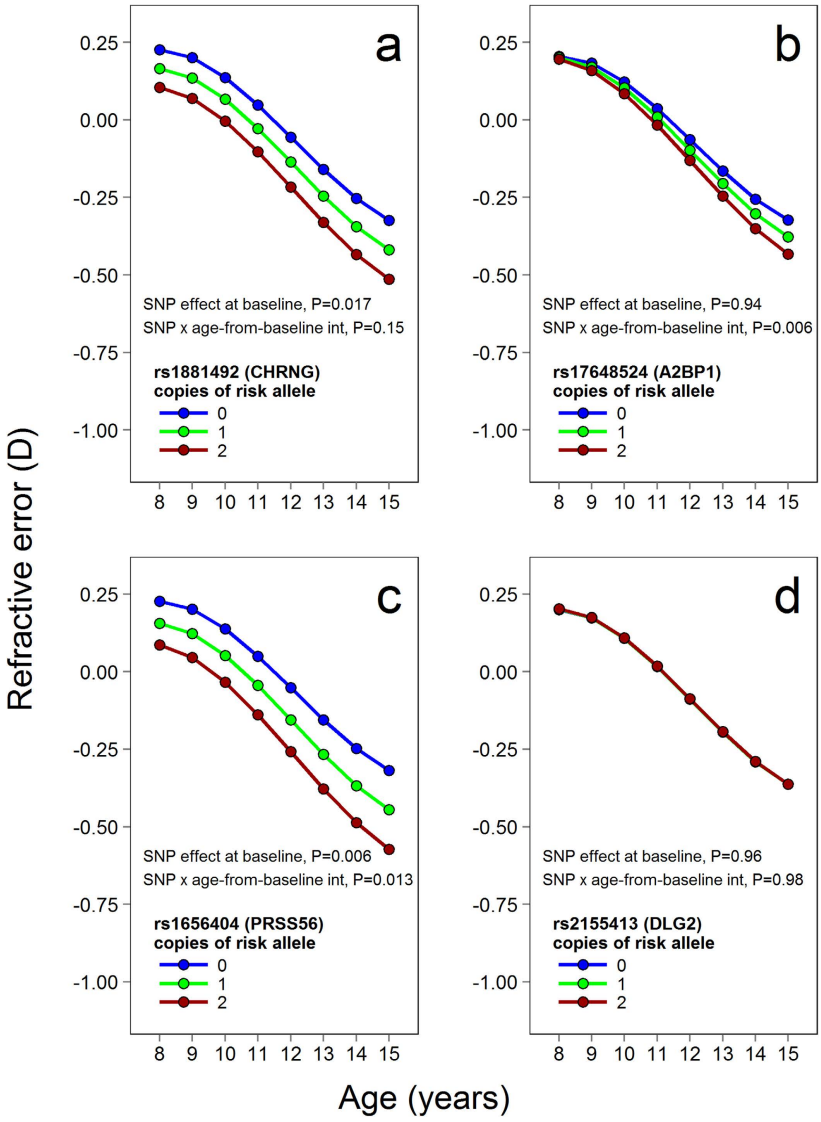

Figure 1. SNPs associated with early-onset and later-onset effects on refractive development during childhood. Analyses were carried out using data from longitudinal eye examinations in 5,200 ALSPAC participants. Each panel shows how refractive error trajectory varied with SNP genotype, for 4 different SNPs: rs1881492, rs17648524, rs1656404 and rs2155413. The lines in each panel show the refractive error trajectories predicted by the best-fit linear mixed model (LMM) for participants carrying the number of risk alleles indicated (0, 1 or 2). The SNPs in panels (**a**,**c**) showed an association with refractive error at baseline, i.e. evidence of early onset in childhood. The SNPs in panels (**b**,**c**) showed an age-dependent interaction with refractive error over later childhood. The SNP in panel (**d**) did not show evidence of effects during childhood.

alleles observed (Fig. 2). At age 7.5 years the genetic risk score explained 0.6% of the variation in refractive error (N = 4,566; P = 6.6E–08); at age 15 years the corresponding figure was 2.3% (N = 3,666; P = 6.9E–21).

For validation we tested the genetic risk score and 12 of the 16 above SNPs (4 were nearly monomorphic in Asians) in the 8 multi-ethnic cross-sectional study cohorts (combined N = 5,599; Table 1). The average age of the participants varied from 6.6 years-old in the STARS cohort to 20.0 years-old in RAINE. The genetic risk score and 4 SNPs – rs7744813 (KCNQ5), rs7837791 (*TOX*), rs8000973 (*ZIC2*) and rs17648524 (*A2BP1*) – were associated with refractive error ($P < 0.05$; Table 3). All 4 SNPs had the expected direction of effect and none exhibited evidence of between-cohort heterogeneity. Interestingly, 3 of the 4 SNPs had evidence of both early-onset and later progressive effects in the discovery cohort. Meta-analysis summary plots for the genetic risk score and the individual SNPs tested for replication are presented in Figure S1. There was suggestive evidence that SNPs had larger effect sizes in Asian than in European ancestry participants (Figure S2).

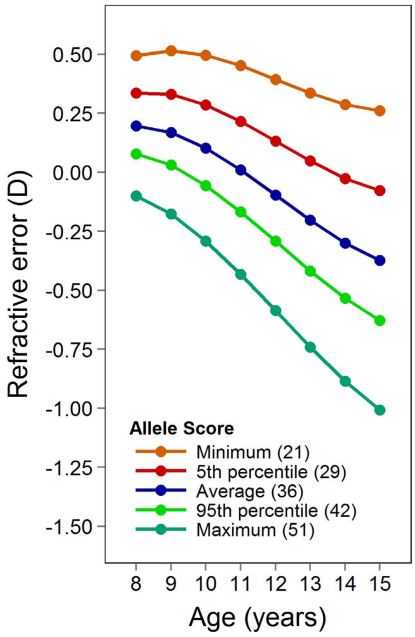

Figure 2. Association between a genetic risk score for 39 SNPs and refractive error trajectories in ALSPAC participants. The genetic risk score was calculated as the sum of the number of risk alleles (0–2) carried by an individual at each of the 39 myopia-susceptibility SNPs. The coloured lines show the trajectories for children carrying the number of risk alleles indicated, as predicted by the best-fit linear mixed model.

Tests in the Discovery Cohort for SNP x SNP interactions for all 741 possible pairs of the 39 SNPs revealed no evidence for interactions exceeding that expected by chance (not shown).

Interactions with time engaged in nearwork. Two types of interactions between SNP genotype and nearwork exposure were evaluated in the ALSPAC discovery cohort: An interaction already present at the baseline age of 7.5 years-old (a 2-way SNP x nearwork interaction) and an interaction that developed progressively during later childhood (a 3-way, SNP x nearwork x age-from-baseline interaction). For a SNP with a risk allele frequency of 0.25, and ignoring the repeated measures nature of the data, the analysis of ALSPAC participants had >90% power to detect an interaction effect of 0.25 D at $\alpha = 0.05$ (and >50% power at $\alpha = 1.28E{-}3$, corresponding to a Bonferroni correction for testing all 39 SNPs).

Nominal support for 3-way SNP x nearwork x age-from-baseline interactions was observed for 4 markers (Fig. 3a–d): rs17428076 upstream of *DLX1* (P = 0.049), rs7829127 within *ZMAT4* (P = 6.3E–04), rs7084402 upstream of *BICC1* (P = 0.043) and rs17648524 within *A2BP1* (P = 2.3E–03). In models that considered just 2-way interactions at baseline, only rs1254319 upstream of *SIX6* showed nominal evidence of an interaction (P = 0.042; Fig. 3e). Of these 5 interactions, only that involving rs7829127 (*ZMAT4*) survived correction for multiple testing (corrected P = 0.025). Consistent with the limited evidence for individual SNP x nearwork interactions, no evidence of interaction between the genetic risk score and ALSPAC children's level of nearwork was observed (2-way interaction, P = 0.20; 3-way interaction, P = 0.086).

Four of the cross-sectional study cohorts, 1 of European ancestry (TEDS) and 3 of Asian ancestry (GZT, SCORM and STARS), had information available regarding the time participants spent engaged in nearwork (Table S6), allowing tests for replication. In the meta-analysis of all 4 replication studies (Table S3) none of the SNPs that showed nominal evidence of an interaction with nearwork in the ALSPAC discovery cohort showed evidence of replication (all P > 0.16). Likewise, the genetic risk score did not show evidence of an interaction with nearwork in the cross-sectional cohorts (P = 0.49).

Interactions with time spent outdoors. In the discovery cohort, only rs13091182 within *ZBTB38* showed nominal evidence of a 3-way interaction involving time outdoors (uncorrected P = 0.028; corrected P > 0.05; Fig. 3f). Surprisingly, the risk allele of rs13091182 was associated with *slower* progression towards myopia (or less hyperopia) in general and with faster progression towards myopia in children who spent *more* time outdoors, suggesting a potentially false-positive result. There was no evidence for 2-way SNP x time outdoors interactions (uncorrected P > 0.20 for all 39 SNPs). Similarly, for the genetic risk score, there was no indication of an interaction with time spent outdoors (2-way interaction, P = 0.16; 3-way interaction, P = 0.49).

Five of the cross-sectional samples had information available on the time participants spent outdoors (TEDS, RAINE, GZT, SCORM and STARS). The single SNP, rs13091182, showing evidence of an interaction with time outdoors in the discovery cohort showed no evidence of replication (indeed, none of the 31 SNPs with

				Europeans (N = 3,143)				Asians (N = 2,456)				Europeans + Asians (N = 5,599)				
Marker	**Chr**	**Gene**	**RA**	***RAF***	**Beta**	**SE**	**P**	***RAF****	**Beta**	**SE**	**P**	**I^2**	**Het_P**	**Beta**	**SE**	**P**
GR Score	–	–	–	–	**−0.026**	**0.007**	**3.8E−04**	–	**−0.048**	**0.011**	**1.4E−05**	0.57	**0.023**	**−0.034**	**0.006**	**1.4E−08**
rs1652333	1	*CD55*	G	0.32	0.042	0.042	0.315	0.52	−0.101	0.056	0.073	0.27	0.210	−0.004	0.034	0.899
rs1881492	2	*CHRNG*	T	0.23	−0.001	0.054	0.986	0.12	0.197	0.102	0.054	0.00	0.926	0.033	0.048	0.483
rs7744813	6	*KCNQ5*	A	0.59	**−0.110**	**0.042**	**0.008**	0.81	0.001	0.071	0.993	0.41	0.107	**−0.083**	**0.036**	**0.021**
rs7837791	8	*TOX*	G	0.53	0.011	0.040	0.772	0.53	**−0.185**	**0.055**	**0.001**	0.49	0.059	**−0.063**	**0.032**	**0.049**
rs4237036	8	*CHD7*	T	0.66	−0.077	0.041	0.062	0.74	0.102	0.069	0.140	0.40	0.112	−0.033	0.035	0.358
rs7042950	9	*RORB*	G	0.22	0.041	0.047	0.391	0.74	−0.004	0.070	0.956	0.00	0.903	0.020	0.039	0.618
rs6480859	10	*KCNMA1*	T	0.37	−0.022	0.041	0.579	0.16	**−0.229**	**0.074**	**0.002**	0.52	0.042	−0.063	0.036	0.075
rs8000973	13	*ZIC2*	C	0.52	−0.067	0.040	0.093	0.21	−0.092	0.070	0.190	0.00	0.470	**−0.081**	**0.035**	**0.019**
rs66913363	14	*BMP4*	G	0.51	−0.021	0.044	0.628	0.73	0.061	0.066	0.354	0.00	0.790	0.002	0.037	0.953
rs524952	15	*GJD2*	A	0.46	−0.008	0.041	0.839	0.48	**−0.171**	**0.057**	**0.003**	0.53	**0.036**	−0.064	0.033	0.058
rs17648524	16	*A2BP1*	C	0.33	**−0.143**	**0.042**	**7.2E−04**	0.06	−0.140	0.106	0.186	0.49	0.057	**−0.146**	**0.039**	**2.0E−04**
rs2969180	17	*SHISA6*	A	0.35	0.028	0.042	0.499	0.51	−0.036	0.056	0.521	0.00	0.553	0.003	0.033	0.926

Table 3. Replication meta-analysis results for SNP main effects. SNPs associated with refractive error in the ALSPAC age-of-onset analyses were tested for association with refractive error in 8 independent cohorts of children (5 European ancestry, 3 Asian ancestry). Abbreviations: Chr = Chromosome. GR Score = Genetic risk score. RA = Risk allele. RAF = Risk allele frequency. *SNPs with minor allele frequencies <0.05 were not examined due to low statistical power.

MAF > 0.05 in both ancestry groups showed evidence of an interaction with time outdoors; all P > 0.17; Table S4). Similarly, the genetic risk score did not show evidence of an interaction with time spent outdoors in the replication cohorts.

Pathway analysis. Pathway analysis identified a single functional pathway for the set of 10 genes (Table 2) implicated in having early-onset effects, namely "*hedgehog signalling*" (Panther P = 0.043; key genes *ZIC2* and *BMP4*). The set of 11 genes implicated in having later-onset effects did not show enrichment for specific pathways.

Discussion

Early-onset and later-onset SNP effects. Sixteen SNPs showed evidence of effects in childhood in ALSPAC participants (Table 2); 10 SNPs had early-onset effects manifest by age 7.5 years, 11 SNPs had later-onset effects, and 5 SNP had early-onset effects that progressed further during later childhood. For the 12 of these 16 SNPs available in the cross-sectional cohorts, 4 showed evidence of replication (Table 3). There was suggestive evidence that SNP effect sizes were approximately 2 times larger in Asian as compared to European ancestry children/adolescents (Figure S2). A genetic risk score that captured the effects of all 39 GWAS-identified variants confirmed the involvement of genetic influences acting at an early age (7.5 years) and then increasing further in magnitude across later childhood.

We sought to discover whether the early-onset and later-onset variants clustered according to functional pathway (for example, if GWAS SNPs A and B are causal variants that affect the expression levels of genes X and Y, respectively, and X acts downstream of Y to regulate refractive development, then one might expect the onset age for SNPs A and B to coincide). However, as summarised in Table 4, SNPs associated with early-onset or later-onset effects did not clearly cluster according to the known function(s) of the genes implicated in mediating the SNPs' effects. Pathway analysis confirmed this impression, with only a single functional pathway being identified. Potential reasons for this lack of functional clustering are, first, that many genes in the genome have diverse functions, which are sometimes poorly understood. For instance, during development of the human visual system, an ion channel may play a vital role during early embryonic development of the retina, be a necessary component of the visual cycle, and yet also contribute to neuronal plasticity. Second, precisely which gene or genes mediate the effect of a specific GWAS-identified SNP is not known with certainty for any of the refractive error GWAS SNPs identified to date: While the nearest gene to a GWAS SNP is usually considered the most likely to be involved, this does not always hold true[35].

The 39 SNPs examined were identified in adult GWAS meta-analyses with sample sizes of approximately 45,000 individuals, and all had small effects (typically 0.1 D per copy of the risk allele). The ALSPAC longitudinal cohort (N = 5,200) had ~80% power to detect an association for a SNP with an effect size of 0.1 D and MAF of 0.25 (but note that the true power would likely have been lower because: refractive development would not be complete by 15 years of age, our models tested primarily for yearly effects rather than cumulative effects, and the "winner's curse" phenomenon[36], i.e. the over-estimation of effect sizes in the original GWAS investigations). Therefore, a likely reason why some of the 39 SNPs we studied failed to show childhood-onset associations in the longitudinal cohort is limited statistical power. Thus, we *cannot* conclude that the SNPs that did not show observable childhood-onset associations have an age-of-onset beyond 15 years-old even though they might well do: much larger studies will be required to definitively address this issue. Similarly, the limited concordance between the longitudinal and cross-sectional studies was also likely due to limited statistical power, although 8 of the 12 SNPs tested for replication showed the expected direction of effect (Table 3).

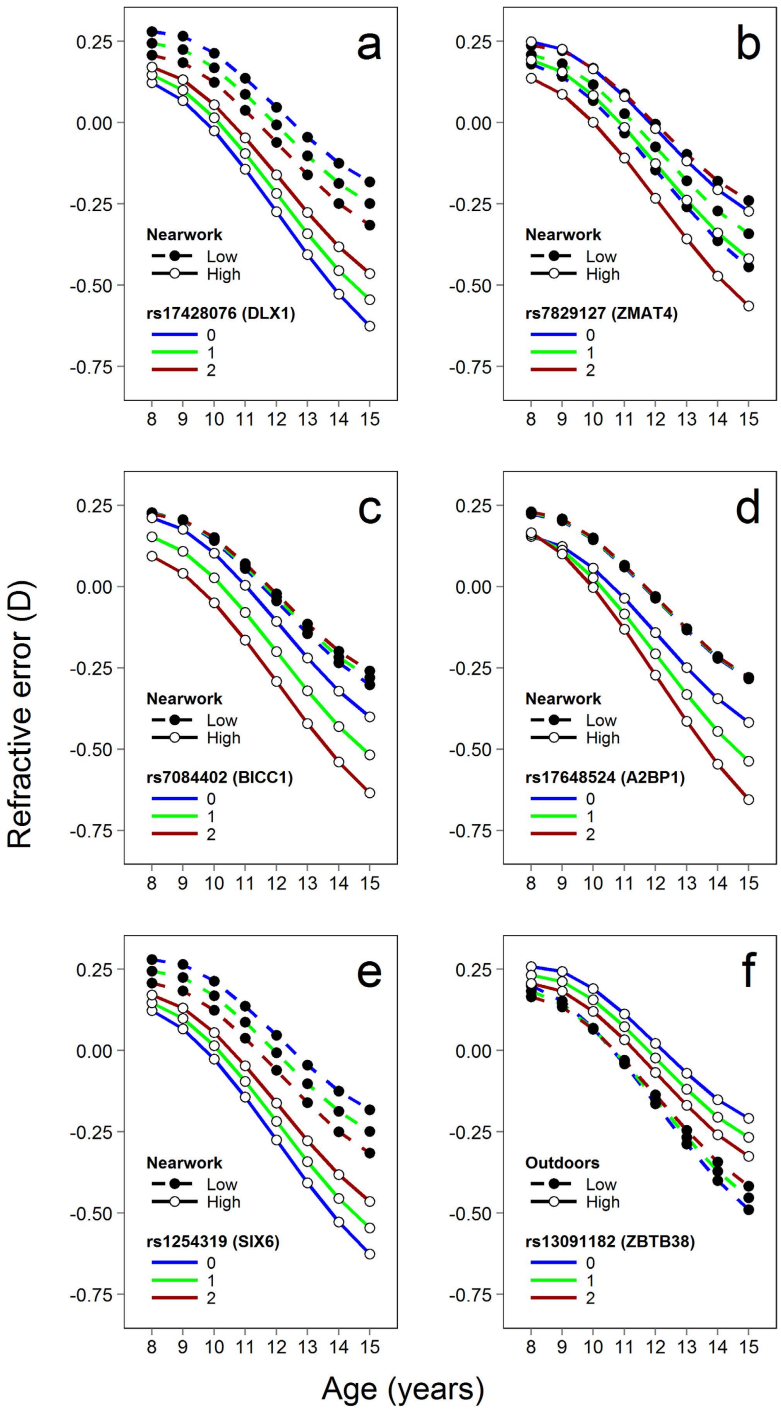

Figure 3. Refractive error trajectories in ALSPAC participants for SNPs showing evidence of an interaction with nearwork or time outdoors. Levels of nearwork activity and time spent outdoors were assessed at 8–9 years of age and classified as high or low (above or below the median level). Panels (**a–d**) show how refractive error trajectories varied depending on nearwork level and the number of risk alleles (0–2) carried for 4 different markers that showed SNP x nearwork x age-from-baseline (3-way) interactions. Panel (**e**) Refractive trajectories for the only marker to show a SNP x nearwork (2-way) interaction at baseline age. Panel (**f**) Refractive trajectories for the only marker to show a SNP x time outdoors x age-from-baseline (3-way) interaction. The coloured lines show the trajectories predicted by the best-fit linear mixed model for children carrying the number of copies of the risk allele indicated in the legend.

SNP	Gene	Role	Longitudinal Early-onset	Longitudinal Later-onset	Cross-sectional	Interaction
GR score	–	–	Y	Y	Y	
rs7837791	TOX	ED	Y	Y	Y	
rs4237036	CHD7	ED		Y		
rs7084402	BICC1	ED				NW
rs8000973	ZIC2	ED	Y	Y	Y	
rs66913363	BMP4	ED	Y			
rs1254319	SIX6	ED				NW
rs1656404	PRSS56	ED, EM	Y	Y		
rs17428076	DLX1	ED,NP				NW
rs12205363	LAMA2	EM	Y			
rs1652333	CD55	IT		Y		
rs1881492	CHRNG	IT	Y			
rs14165	CACNA1D	IT	Y			
rs6480859	KCNMA1	IT		Y		
rs7744813	KCNQ5	IT, VC	Y	Y	Y	
rs17648524	A2BP1	NP		Y	Y	NW
rs13091182	ZBTB38	U				TO
rs9307551	LOC100506035	U				NW
rs7829127	ZMAT4	U				NW
rs2969180	SHISA6	U	Y	Y		
rs7042950	RORB	VC		Y		
rs10882165	CYP26A1	VC	Y			
rs524952	GJD2	VC		Y		

Table 4. Summary of findings. SNPs with evidence ($P < 0.05$) of early-onset, later onset, or GxE interaction effects on refractive error in one or more analysis are highlighted. Abbreviations: Y = Yes, NW = Nearwork, TO = Time outdoors, VC = Visual cycle, NP = Neuronal plasticity, IT = Ion transport, EM = Extracellular matrix, ED = Eye development, U = Unknown.

Interactions with environmental exposures. In general there was scant evidence for GxE interactions, especially for SNP x time spent outdoors effects. Given the expected power of >90% to detect interaction effects with a magnitude 0.25 D or more, this argues against SNP x nearwork or SNP x time outdoors interactions of this size being present for the majority of variants studied, rather than lack of statistical power precluding their discovery.

In the ALSPAC longitudinal analysis the gene-environment interaction between *ZMAT4* SNP rs7829127 genotype and nearwork survived correction for multiple-testing ($P_{corr} = 0.025$). Although this interaction was not replicated in the cross-sectional meta-analyses, variants at this locus have previously been reported to show an interaction with the duration of education in a meta-analysis of 5 studies from Singapore (SNP x education interaction = −0.42 D, 95% C.I. −0.15 to −0.69, $P = 0.002$)[37]. We did not explore interactions between SNPs and years of education, since in several cohorts the participants were still students. The functional role of *ZMAT4* is not known.

Why might GxE interactions involving these 39 SNPs be so scarce? First, differences in environmental risk exposures were not considered in the original GWAS investigations carried out by CREAM[9] and 23andMe[8]. Thus, SNPs with strong interaction effects but no main effects may not have been detected using those GWAS designs. Second, the age range and ethnic diversity of the original GWAS discovery samples were highly varied. Given the substantial increase in the prevalence of myopia in the past few decades, which strongly implicates a major role for environmental risk factors, it seems almost certain that the individuals studied in the CREAM and 23andMe GWAS meta-analyses would have grown up in environments with a wide range of risk exposure profiles depending on the participants' years of birth: young (recently born) individuals would have been exposed to a much more myopiagenic environment than older (more distantly born) adults. Therefore, a variant that increases the risk of myopia only in children who perform excessive nearwork may have shown an (apparent) main effect association with refractive error in a GWAS carried out in a young adult cohort, in which participants were ubiquitously exposed to high nearwork during childhood. However, this same variant may not have shown an association with refractive error in a GWAS on an older cohort, due to the lower nearwork exposure during childhood of the older individuals. Thus, support for the association of such a variant in the CREAM and 23andMe GWAS samples may have been diluted rather than strengthened during the meta-analysis of younger and older cohorts.

Separate from tests for gene-environment interactions, time spent outdoors itself *was not* associated with myopia in 3 of the 5 cross-sectional studies (GZT, STARS, and TEDS) and the association was of borderline significance in another (TEDS). This lack of an association with time outdoors implies that detecting a SNP x time outdoors interaction would also have been challenging, even after meta-analysis of data from all 5 cohorts.

Interestingly, a large-effect GxE interaction predisposing children to myopia was identified recently, involving a rare variant at the *APLP2* gene locus and time spent reading[38]. *APLP2* was implicated in myopia

development through studies in an animal model[39], which – given the statistical challenge of identifying GxE interaction effects in human populations – suggests that combining findings from animal models and human studies could be a fruitful future approach.

We reasoned that correction for multiple testing *was not* appropriate when examining the age-of-onset of the 39 SNPs investigated, because of compelling existing evidence that by adulthood these SNPs truly are associated with refractive error. That is, our analyses sought to discover whether or not each SNP had an effect during childhood, not whether a group of candidate SNPs were associated with refractive error *per se*. By contrast, in view of very limited evidence for interactions with environmental exposures for most of the SNPs examined, correction for multiple testing *was* considered appropriate when evaluating SNP x nearwork and SNP x time outdoors interactions: In these analyses, a large number of independent hypothesis tests were carried out, with little or no prior knowledge that an interaction must be present at some age.

Limitations of the present work. The present work had a number of other limitations. The cross-sectional samples were not matched for age, which prevented us from testing for "early" and "later" onset effects in the replication stage. The level of exposure to nearwork and time outdoors also varied across samples, which meant that imprecisely-matched interaction effects were meta-analysed, potentially reducing statistical power. We chose to categorise time spent performing nearwork and time spent outdoors relative to the median activity level in each study sample because the measurement scales used in the various studies were not standardised (precluding the use of an absolute measure). If in reality these environmental risk factors exert their influence non-linearly – for instance if spending more than a certain threshold number of hours per day outdoors is needed to protect against myopia development – then our approach may have poorly captured the effects of the environmental exposures. For the combined meta-analysis of European and Asian cross-sectional studies, we assumed that each lead SNP tagged the underlying causal variant(s) equally well in European and Asian ancestry individuals, which is an oversimplification. Finally, we chose to examine only a simple, binary GxE model, whereas more complex scenarios may exist[40–42].

Conclusions

Specific myopia-predisposing SNPs were found to differ in the age at which they had their effects, and whether or not these effects got progressively stronger during later childhood. Thus, SNPs implicating the genes *CHRNG*, *CACNA1D*, *LAMA2*, *CYP26A1* and *BMP4* were associated with early onset changes in refractive error that did not progress further, while SNPs close to *PRSS56*, *KCNQ5*, *TOX*, *ZIC2 and SHISA6* showed early-onset effects that became greater still at older ages. Effects that only appeared in later childhood – after the age of 7.5 years – implicated the genes *CD55, CHD7*, *RORB*, *KCNMA1*, *A2BP1* and *GJD2*. Gene-environment interactions involving nearwork or time outdoors were rare or absent for the vast majority of the GWAS-identified SNPs, and indeed a genetic risk score that demonstrated very convincing association with early-onset ($P = 2.2E{-}9$) and later progressive ($P = 5.8E{-}14$) changes in refractive error appeared to act independently of the time children spent in these activities. However, one robust interaction between rs7829127 in *ZMAT4* and time spent performing nearwork (nominal $P = 6.3E{-}04$, corrected $P = 0.025$) was observed, replicating a previously-identified interaction involving rs7829127 and years of education[37,43,44].

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Author Contributions

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Additional Information

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Supplementary Information

Childhood gene-environment interactions and age-dependent effects of genetic variants associated with refractive error and myopia: The CREAM Consortium

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Index

Table S1. SNPs examined.	3
Table S2. Age-of-onset of SNP associations in Discovery cohort (ALSPAC).	4
Table S3. Meta-analysis of SNP x near work interaction effects in cross-sectional cohorts.	5
Table S4. Meta-analysis of SNP x time outdoors interaction effects in cross-sectional cohorts.	6
Table S5. Genotyping and imputation details.	7
Table S6. Near work exposure in each cohort.	8
Table S7. Time outdoors exposure in each cohort.	9
Table S8. Refraction details for the ALSPAC discovery cohort.	10
Figure S1. Meta-analysis summary plots for cross-sectional cohorts.	11
Figure S2. SNP effects in European and Asian meta-analysis samples.	15
Figure S3. SNP x nearwork interactions at ages 7 and 12 in the ALSPAC discovery cohort.	16
Recruitment of participants and phenotypic assessment.	17
Supplementary references	22

See Appendix 3.4

Table S1. SNPs examined.

SNP	Chr	Pos	Gene	Citation
rs1652333	1	207470460	*CD55*	Verhoeven et al. 2013
rs4373767	1	219759682	*ZC3H11B*	Cheng et al. 2013
rs17412774	2	146773948	*PABPCP2*	Kiefer et al. 2013
rs17428076	2	172851936	*DLX1*	Kiefer et al. 2013
rs1898585	2	178660450	*PDE11A*	Kiefer et al. 2013
rs1656404	2	233379941	*PRSS56*	Verhoeven et al. 2013
rs1881492	2	233406998	*CHRNG*	Verhoeven et al. 2013
rs14165	3	53847408	*CACNA1D*	Verhoeven et al. 2013
rs13091182	3	141133960	*ZBTB38*	Kiefer et al. 2013
rs9307551	4	80530671	*LOC100506035*	Verhoeven et al. 2013
rs5022942	4	81959966	*BMP3*	Kiefer et al. 2013
rs7744813	6	73643289	*KCNQ5*	Verhoeven et al. 2013
rs12205363	6	129834628	*LAMA2*	Verhoeven et al. 2013
rs7829127	8	40726394	*ZMAT4*	Verhoeven et al. 2013
rs7837791	8	60179086	*TOX*	Verhoeven et al. 2013
rs4237036	8	61701057	*CHD7*	Verhoeven et al. 2013
rs11145465	9	70989531	*TJP2*	Verhoeven et al. 2013
rs7042950	9	77149837	*RORB*	Verhoeven et al. 2013
rs7084402	10	60265404	*BICC1*	Verhoeven et al. 2013
rs6480859	10	79081948	*KCNMA1*	Kiefer et al. 2013
rs745480	10	85986554	*RGR*	Kiefer et al. 2013
rs10882165	10	94924324	*CYP26A1*	Verhoeven et al. 2013
rs1381566	11	40149607	*LRRC4C*	Kiefer et al. 2013
rs2155413	11	84634790	*DLG2*	Kiefer et al. 2013
rs11601239	11	105556598	*GRIA4*	Verhoeven et al. 2013
rs3138144	12	56114768	*RDH5*	Verhoeven et al. 2013
rs12229663	12	71249996	*PTPRR*	Verhoeven et al. 2013
rs8000973	13	100691367	*ZIC2*	Verhoeven et al. 2013
rs2184971	13	100818092	*PCCA*	Verhoeven et al. 2013
rs66913363	14	54413001	*BMP4*	Kiefer et al. 2013
rs1254319	14	60903757	*SIX6*	Verhoeven et al. 2013
rs524952	15	35005885	*GJD2*	Verhoeven et al. 2013
rs4778879	15	79372875	*RASGRF1*	Verhoeven et al. 2013
rs17648524	16	7459683	*A2BP1*	Verhoeven et al. 2013
rs2969180	17	11407901	*SHISA6*	Verhoeven et al. 2013
rs17183295	17	31078272	*MYO1D*	Verhoeven et al. 2013
rs4793501	17	68718734	*KCNJ2*	Verhoeven et al. 2013
rs12971120	18	72174023	*CNDP2*	Verhoeven et al. 2013
rs235770	20	6761765	*BMP2*	Verhoeven et al. 2013

Table S2. Age-of-onset of SNP associations in discovery cohort (ALSPAC).

					SNP main effect at baseline (D)			SNP x Age interaction (D/yr)		
Marker	**Chr**	**Gene**	**RA**	**RAF**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**
GR Score	-	-	-	-	**-0.018**	**0.003**	**2.2E-09**	**-0.003**	**0.000**	**5.8E-14**
rs1652333	1	*CD55*	G	0.32	-0.002	0.019	9.3E-01	**-0.005**	**0.003**	**4.0E-02**
rs4373767	1	*ZC3H11B*	T	0.38	-0.005	0.018	8.0E-01	-0.001	0.003	7.9E-01
rs17412774	2	*PABPCP2*	A	0.57	-0.026	0.018	1.5E-01	-0.004	0.003	1.7E-01
rs17428076	2	*DLX1*	C	0.74	-0.026	0.021	2.1E-01	0.000	0.003	8.7E-01
rs1898585	2	*PDE11A*	T	0.17	0.005	0.025	8.3E-01	-0.006	0.003	1.1E-01
rs1656404	2	*PRSS56*	A	0.21	**-0.066**	**0.024**	**5.7E-03**	**-0.008**	**0.003**	**1.3E-02**
rs1881492	2	*CHRNG*	T	0.23	**-0.058**	**0.024**	**1.7E-02**	-0.005	0.003	1.5E-01
rs14165	3	*CACNA1D*	G	0.70	**-0.040**	**0.020**	**4.2E-02**	-0.001	0.003	7.7E-01
rs13091182	3	*ZBTB38*	G	0.67	-0.032	0.019	8.4E-02	0.001	0.003	6.4E-01
rs9307551	4	*LOC100506035*	A	0.20	-0.026	0.022	2.4E-01	-0.005	0.003	1.3E-01
rs5022942	4	*BMP3*	A	0.22	-0.003	0.021	8.7E-01	-0.004	0.003	1.8E-01
rs7744813	6	*KCNQ5*	A	0.59	**-0.048**	**0.019**	**9.9E-03**	**-0.005**	**0.003**	**3.5E-02**
rs12205363	6	*LAMA2*	T	0.92	**-0.097**	**0.035**	**5.7E-03**	-0.008	0.005	1.2E-01
rs7829127	8	*ZMAT4*	A	0.75	-0.006	0.022	7.7E-01	0.002	0.003	4.2E-01
rs7837791	8	*TOX*	G	0.53	**-0.045**	**0.018**	**1.1E-02**	**-0.005**	**0.002**	**2.7E-02**
rs4237036	8	*CHD7*	T	0.66	0.020	0.019	2.9E-01	**-0.007**	**0.003**	**5.6E-03**
rs11145465	9	*TJP2*	A	0.21	-0.036	0.021	9.6E-02	-0.004	0.003	2.4E-01
rs7042950	9	*RORB*	G	0.22	0.018	0.022	4.1E-01	**-0.009**	**0.003**	**2.5E-03**
rs7084402	10	*BICC1*	G	0.49	-0.019	0.018	3.0E-01	-0.001	0.003	7.7E-01
rs6480859	10	*KCNMA1*	T	0.37	-0.029	0.018	1.1E-01	**-0.008**	**0.002**	**1.3E-03**
rs745480	10	*RGR*	G	0.48	-0.021	0.018	2.3E-01	-0.003	0.002	2.6E-01
rs10882165	10	*CYP26A1*	T	0.40	**-0.035**	**0.018**	**4.8E-02**	0.001	0.003	7.6E-01
rs1381566	11	*LRRC4C*	G	0.18	-0.023	0.026	3.8E-01	-0.002	0.004	5.6E-01
rs2155413	11	*DLG2*	A	0.45	0.001	0.018	9.6E-01	0.000	0.002	9.8E-01
rs11601239	11	*GRIA4*	C	0.49	0.004	0.018	8.0E-01	-0.001	0.002	6.9E-01
rs3138144	12	*RDH5*	G	0.54	-0.027	0.021	1.9E-01	-0.002	0.003	5.2E-01
rs12229663	12	*PTPRR*	A	0.76	-0.033	0.022	1.3E-01	0.000	0.003	8.8E-01
rs8000973	13	*ZIC2*	C	0.52	**-0.042**	**0.018**	**1.8E-02**	**-0.008**	**0.002**	**1.5E-03**
rs2184971	13	*PCCA*	A	0.60	0.002	0.018	8.9E-01	0.000	0.002	9.1E-01
rs66913363	14	*BMP4*	G	0.51	**-0.051**	**0.018**	**5.3E-03**	0.001	0.003	7.2E-01
rs1254319	14	*SIX6*	A	0.29	-0.011	0.020	5.8E-01	-0.002	0.003	3.8E-01
rs524952	15	*GJD2*	A	0.46	-0.018	0.018	3.3E-01	**-0.008**	**0.003**	**8.8E-04**
rs4778879	15	*RASGRF1*	G	0.42	-0.017	0.018	3.7E-01	-0.004	0.003	9.4E-02
rs17648524	16	*A2BP1*	C	0.33	-0.001	0.019	9.4E-01	**-0.007**	**0.003**	**5.6E-03**
rs2969180	17	*SHISA6*	A	0.35	**-0.039**	**0.019**	**3.9E-02**	**-0.005**	**0.003**	**4.9E-02**
rs17183295	17	*MYO1D*	T	0.19	0.006	0.023	7.8E-01	-0.004	0.003	1.5E-01
rs4793501	17	*KCNJ2*	T	0.53	0.000	0.018	9.8E-01	-0.002	0.003	4.2E-01
rs12971120	18	*CNDP2*	A	0.82	0.017	0.021	4.1E-01	-0.003	0.003	3.2E-01
rs235770	20	*BMP2*	T	0.37	-0.010	0.019	5.8E-01	-0.005	0.003	5.3E-02

Abbreviations: Chr=Chromosome. RA=Risk allele. RAF=Risk allele frequency.

Table S3. Meta-analysis of SNP x near work interaction effects in cross-sectional cohorts. Beta shows the difference in refractive error (D) associated with each copy of the risk allele in individuals exposed to high versus low levels of nearwork. Meta-analysis was conducted for 4 cohorts (TEDS, GZT, SCORM and STARS) combined N=3,154.

SNP	Chr	Gene	RA	Beta	SE	P		I^2	$P_{Q\text{-test}}$
Allele score	-	-	A	-0.014	0.021	0.489		0	0.584
rs1652333	1	*CD55*	G	-0.049	0.108	0.649		0	0.460
rs4373767	1	*ZC3H11B*	T	-0.217	0.116	0.061		0	0.979
rs17412774	2	*PABPCP2*	A	0.157	0.114	0.169		0	0.877
rs1898585	2	*PDE11A*	T	-0.189	0.117	0.108		0	0.769
rs1881492	2	*CHRNG*	T	0.253	0.185	0.170		0	0.609
rs9307551	4	*LOC100506035*	A	**-0.237**	**0.113**	**0.035**		9	0.348
rs5022942	4	*BMP3*	A	-0.088	0.117	0.450		0	0.621
rs7744813	6	*KCNQ5*	A	0.251	0.134	0.061		0	0.856
rs7829127	8	*ZMAT4*	A	-0.104	0.166	0.529		55	0.084
rs7837791	8	*TOX*	G	-0.031	0.106	0.771		9	0.351
rs4237036	8	*CHD7*	T	-0.133	0.129	0.304		43	0.152
rs7042950	9	*RORB*	G	0.009	0.133	0.946		0	0.927
rs7084402	10	*BICC1*	G	-0.002	0.108	0.985		0	0.915
rs6480859	10	*KCNMA1*	T	-0.242	0.135	0.073		0	0.832
rs745480	10	*RGR*	G	0.020	0.109	0.854		0	0.712
rs1381566	11	*LRRC4C*	G	-0.060	0.129	0.644		0	0.502
rs2155413	11	*DLG2*	A	0.215	0.138	0.120		28	0.379
rs11601239	11	*GRIA4*	C	-0.008	0.111	0.943		0	0.765
rs3138144	12	*RDH5*	G	-0.083	0.170	0.625		0	0.409
rs12229663	12	*PTPRR*	A	0.042	0.111	0.704		0	0.832
rs8000973	13	*ZIC2*	C	-0.039	0.128	0.759		0	0.581
rs2184971	13	*PCCA*	A	0.091	0.127	0.473		0	0.896
rs66913363	14	*BMP4*	G	0.205	0.125	0.099		0	0.403
rs1254319	14	*SIX6*	A	-0.078	0.120	0.513		0	0.698
rs524952	15	*GJD2*	A	-0.033	0.110	0.761		15	0.317
rs4778879	15	*RASGRF1*	G	0.033	0.110	0.766		0	0.631
rs17648524	16	*A2BP1*	C	0.178	0.176	0.312		22	0.279
rs2969180	17	*SHISA6*	A	0.010	0.108	0.927		0	0.435
rs4793501	17	*KCNJ2*	T	0.047	0.110	0.671		56	0.078
rs12971120	18	*CNDP2*	A	-0.049	0.120	0.682		0	0.581
rs235770	20	*BMP2*	T	-0.031	0.131	0.814		0	0.847

Abbreviations: Chr=Chromosome. RA=Risk allele. I^2=Heterogeneity statistic. $P_{Q\text{-test}}$=P-value for Cochran's Q-test.

Table S4. Meta-analysis of SNP x time outdoors interaction effects in cross-sectional cohorts. Beta shows the difference in refractive error (D) associated with each copy of the risk allele in individuals exposed to high versus low levels of time outdoors. Meta-analysis was conducted for 5 cohorts (TEDS, RAINE, GZT, SCORM and STARS) combined N=3,908.

SNP	Chr	Gene	RA	Beta	SE	P		I^2	$P_{Q\text{-test}}$
Allele score	-	-	A	-0.003	0.019	0.892		29	0.231
rs1652333	1	*CD55*	G	0.108	0.104	0.301		2	0.394
rs4373767	1	*ZC3H11B*	T	0.132	0.104	0.202		0	0.974
rs17412774	2	*PABPCP2*	A	0.064	0.107	0.549		0	0.841
rs1898585	2	*PDE11A*	C	-0.038	0.120	0.754		0	0.706
rs1881492	2	*CHRNG*	G	0.011	0.156	0.946		48	0.101
rs9307551	4	*LOC100506035*	C	0.088	0.110	0.421		0	0.675
rs5022942	4	*BMP3*	G	0.028	0.114	0.804		0	0.550
rs7744813	6	*KCNQ5*	A	-0.097	0.116	0.404		8	0.361
rs7829127	8	*ZMAT4*	A	0.015	0.137	0.915		0	0.951
rs7837791	8	*TOX*	T	-0.032	0.099	0.746		0	0.528
rs4237036	8	*CHD7*	T	-0.081	0.114	0.477		0	0.927
rs7042950	9	*RORB*	A	0.101	0.122	0.411		0	0.708
rs7084402	10	*BICC1*	G	0.009	0.103	0.928		0	0.864
rs6480859	10	*KCNMA1*	C	-0.157	0.113	0.165		0	0.663
rs745480	10	*RGR*	C	-0.070	0.100	0.486		0	0.492
rs1381566	11	*LRRC4C*	T	-0.121	0.141	0.388		23	0.269
rs2155413	11	*DLG2*	A	-0.006	0.113	0.961		33	0.198
rs11601239	11	*GRIA4*	C	0.028	0.102	0.782		0	0.674
rs3138144	12	*RDH5*	G	-0.137	0.149	0.358		14	0.326
rs12229663	12	*PTPRR*	G	-0.045	0.109	0.681		0	0.587
rs8000973	13	*ZIC2*	T	-0.140	0.111	0.205		0	0.698
rs2184971	13	*PCCA*	G	-0.054	0.109	0.623		7	0.366
rs66913363	14	*BMP4*	G	0.016	0.122	0.896		0	0.703
rs1254319	14	*SIX6*	A	0.023	0.110	0.834		23	0.269
rs524952	15	*GJD2*	T	-0.055	0.106	0.606		0	0.829
rs4778879	15	*RASGRF1*	A	0.068	0.104	0.513		52	0.082
rs17648524	16	*A2BP1*	G	0.044	0.129	0.733		0	0.816
rs2969180	17	*SHISA6*	A	0.037	0.103	0.720		0	0.910
rs4793501	17	*KCNJ2*	C	-0.139	0.102	0.174		0	0.672
rs12971120	18	*CNDP2*	A	-0.027	0.116	0.813		6	0.372
rs235770	20	*BMP2*	C	-0.062	0.134	0.642		0	0.648

Abbreviations: Chr=Chromosome. RA=Risk allele. I^2=Heterogeneity statistic. $P_{Q\text{-test}}$=P-value for Cochran's Q-test.

Table S5. Genotyping and imputation details

Study	Genotyping platform	Imputation	Reference population (1000G)	QC
ALSPAC	Illumina HumanHap550	MACH/minimac	GIANT phase1 release v3	Cheng et al. 2013 [1]
BATS/TEST	Illumina HumanHap610/660-Quad	MACH	1000G Phase 1 release on Aug 4, 2010	Yazar et al. 2015 [2]
RAINE	Illumina 660W-Quad	MACH/minimac	1000G Phase 1 release on Nov 23, 2010	Yazar et al. 2015 [2]
TEDS	Affymetrix GeneChip 6.0	IMPUTE2 v2.3.0	1000G Phase 1 release v3	Davis et al. 2014 [3]
TEST	Illumina HumanHap610/660-Quad	MACH	1000G Phase 1 release on Aug 4, 2010	Yazar et al. 2015 [2]
WESDR	Illumina Human Omni1Quad	IMPUTE2 v2.3.0	1000G phase 1 integrated variant set release v3	Hosseini et al. 2015 [4]
Guangzhou Twins	Affymetrix Gene Titan	IMPUTE2 v2.3.0	1000 genomes phase 1 (Nov 2010 release)	-
SCORM	Illumina HumanHap550/550-Duo	MACH/minimac	1000 genomes phase 1 cosmopolitan panel haplotypes (March 2012 release)	Verhoeven et al. 2013
STARS Parents	Illumina HumanHap610-Quad	MACH/minimac	1000 genomes phase 1 cosmopolitan panel haplotypes (March 2012 release)	Verhoeven et al. 2013

Abbreviations: 1000G, One thousand genomes project. QC, Quality control.

Table S6. Time spent performing near work. Abbreviations: NA, Not available for analysis.

Cohort	Instrument	Low	High
ALSPAC	Maternal questionnaire: On normal days in school holidays, how much time on average does your child spend each day reading books for pleasure? (a) None at all, (b) 1 hour, (c) 1–2 hours, (d) 3 or more hours.	<1.0 hrs/dy	≥1.0 hrs/dy
BATS	NA	NA	NA
GZT	Child questionnaire: How many hours per day do you spend doing near work in weekday? How many hours per day do you spend doing near work in weekend? During school terms (February to July, September to December), the average time of each type activity was calculated as (5×weekday + 2×weekend)/7. During holidays, the daily visual activity refers to weekend information. In China, every year has 9 months semester days and 3 months summer/winter holidays. The average nearwork per day in the past year was calculated as (9×semester day time + 3×holiday time)/12.	<4.2 hrs/dy	≥4.2 hrs/dy
RAINE	NA	NA	NA
SCORM	Maternal questionnaire: Q1. In the past year, how many hours per day (outside school hours) did your child spend reading and writing? (a) Weekdays: __ hours/day; (b) At the weekend: __hours/day. Q2. In the past year, how many hours per day (outside of regular school hours) did your child spend watching TV, playing video games, and using a computer? (a) Weekdays: __ hours/day; (b) At the weekend: __hours/day. Total = (1a x 5/7)+(1b x 2/7)+(2a x 5/7)+(2b x 2/7)	<2.7 hrs/dy	≥2.7 hrs/dy
STARS	Maternal questionnaire: Q1. During the school years, how many hours per day (outside of regular school hours) would you estimate your child spends reading and writing (school work & reading for pleasure? (a) Weekdays: __ hours/day; (b) At the weekend: __hours/day. Q2. During the school years, how many hours per day (outside of regular school hours) would you estimate your child spends drawing, watching TV, playing video games, computers, and other near work activity (cutting paper and playing toys etc)? (a) Weekdays: __ hours/day; (b) At the weekend: __hours/day. Total = (1a x 5/7)+(1b x 2/7)+(2a x 5/7)+(2b x 2/7)	<1.2 hrs/dy	≥1.2 hrs/dy
TEDS	Child questionnaire: Which of the following activities do you do, and how much do you enjoy them? If you have never had a go at these activities, please cross Never done. (a) Reading for fun: _ hours per week (b) Computer games: _hours per week Total hours per day = (hours per week (a) + hours per week (b)) / 7	≤ 1.0 hrs/day	> 1.0 hrs/day
TEST	NA	NA	NA
WESDR	NA	NA	NA

Table S7. Time spent outdoors. Abbreviations: NA, Not available for analysis.

Cohort	Instrument	Low	High
ALSPAC	Maternal questionnaire: On a school weekday, how much time on average does your child spend each day out of doors in summer? (a) None at all, (b) 1 hour, (c) 1–2 hours, (d) 3 or more hours.	<3.0 hrs/dy	≥3.0 hrs/dy
BATS	NA	NA	NA
GZT	Child questionnaire: How many hours per day do you spend outdoors in weekday? How many hours per day do you spend outdoors in weekend? During school terms (February to July, September to December), the average time of each type activity was calculated as (5×weekday + 2×weekend)/7. During holidays, the daily visual activity refers to weekend information. In China, every year has 9 months semester days and 3 months summer/winter holidays. The average nearwork per day in the past year was calculated as (9×semester day time + 3×holiday time)/12.	<1.4 hrs/dy	≥1.4 hrs/dy
RAINE	Young adult questionnaire: In the summer, when not working at your job or at school, what part of the day do you spend outside	≤1/4 of the day	>1/4 of the day
SCORM	Maternal questionnaire: How much time does your child spend outside: (a) Plays outdoors (in the backyard, walks, bike riding): __ hours/day (b) Participates in outdoor leisure activities (Family BBQs, park, Picnic, Beach): __ hours/day (c) Outdoor sports: __ hours/day; Total = (a) + (b) + (c)	<3.1 hrs/dy	≥3.1 hrs/dy
STARS	Maternal questionnaire: How much time does your child spend outside: (a) Plays outdoors (in the backyard, walks, bike riding): __ hours/day (b) Participates in outdoor leisure activities (Family BBQs, park, Picnic, Beach): __ hours/day (c) Outdoor sports: __ hours/day; Total = (a) + (b) + (c)	<0.5 hrs/dy	≥0.5 hrs/dy
TEDS	Child questionnaire: Which of the following activities do you do, and how much do you enjoy them? If you have never had a go at these activities, please cross Never done. (a) Hang out with friends outside (eg, in park): _ hours per week Total hours per day = total hours per week (a) / 7	≤0.6 hrs/day	> 0.6 hrs/day
TEST	NA	NA	NA
WESDR	NA	NA	NA

Table S8. Refraction details for the ALSPAC discovery cohort.

Clinic visit	N	Age (95% C.I.) in years	Refraction (95% C.I.) in D
7	4680	7.51 (7.50 to 7.52)	+0.18 (+0.16 to +0.21)
10	4955	10.63 (10.62 to 10.64)	+0.05 (+0.02 to +0.08)
11	4711	11.73 (11.72 to 11.74)	-0.04 (-0.07 to +0.00)
12	4740	12.80 (12.79 to 12.80)	-0.18 (-0.22 to -0.15)
15	3666	15.43 (15.42 to 15.44)	-0.39 (-0.43 to -0.35)

[Next page] **Figure S1. Meta-analysis summary plots for cross-sectional cohorts.** For each cohort, the change in refractive error per copy of the risk allele is shown by a black diamond (black horizontal line shows 95% confidence interval). The meta-analysis result is shown as a large diamond, with blue and red indicating meta-analysis P≥0.05 and P<0.05, respectively. Note that SNPs with MAF<0.05 in Asians were not analysed.

Figure S1 continued:

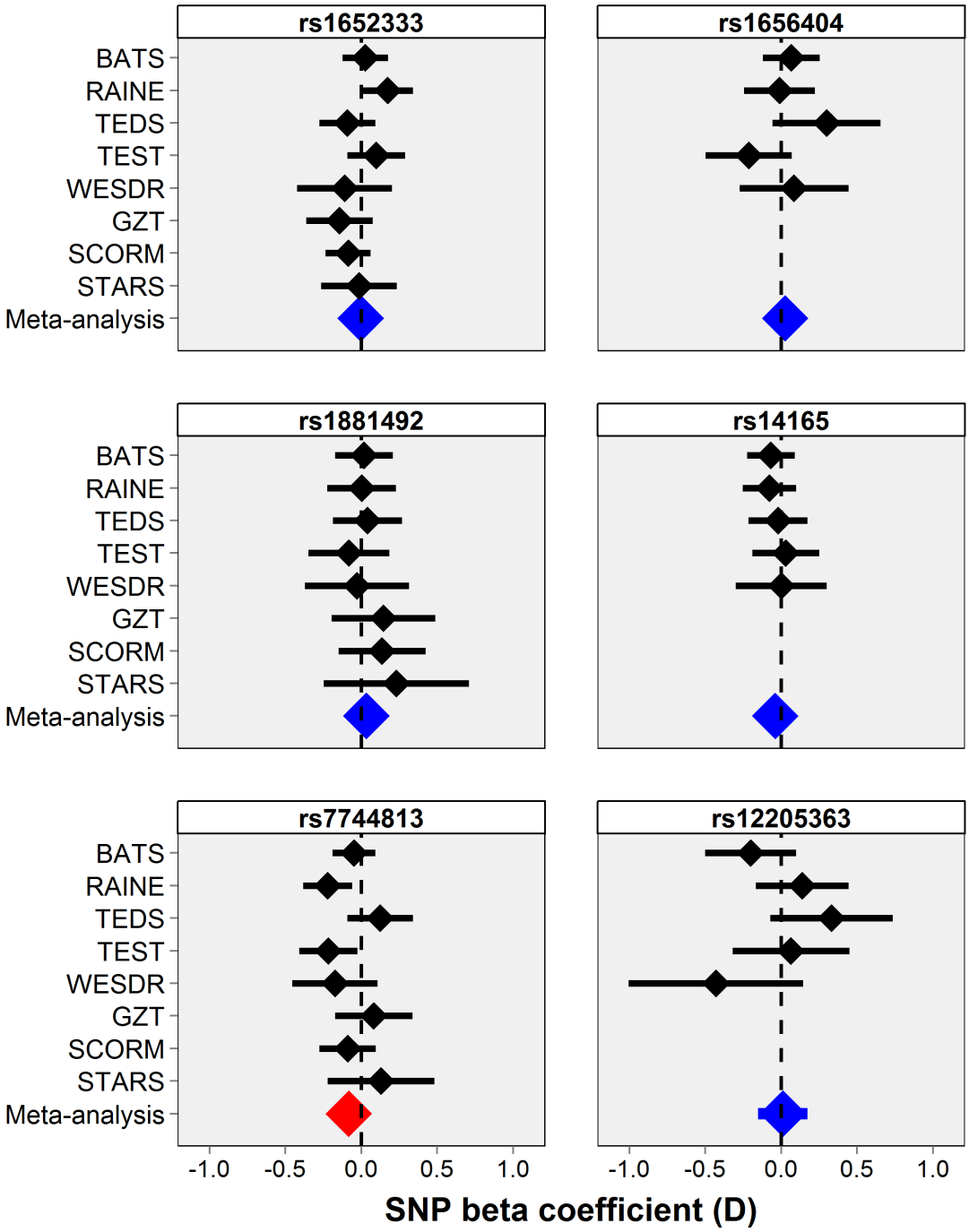


Figure S1 continued:

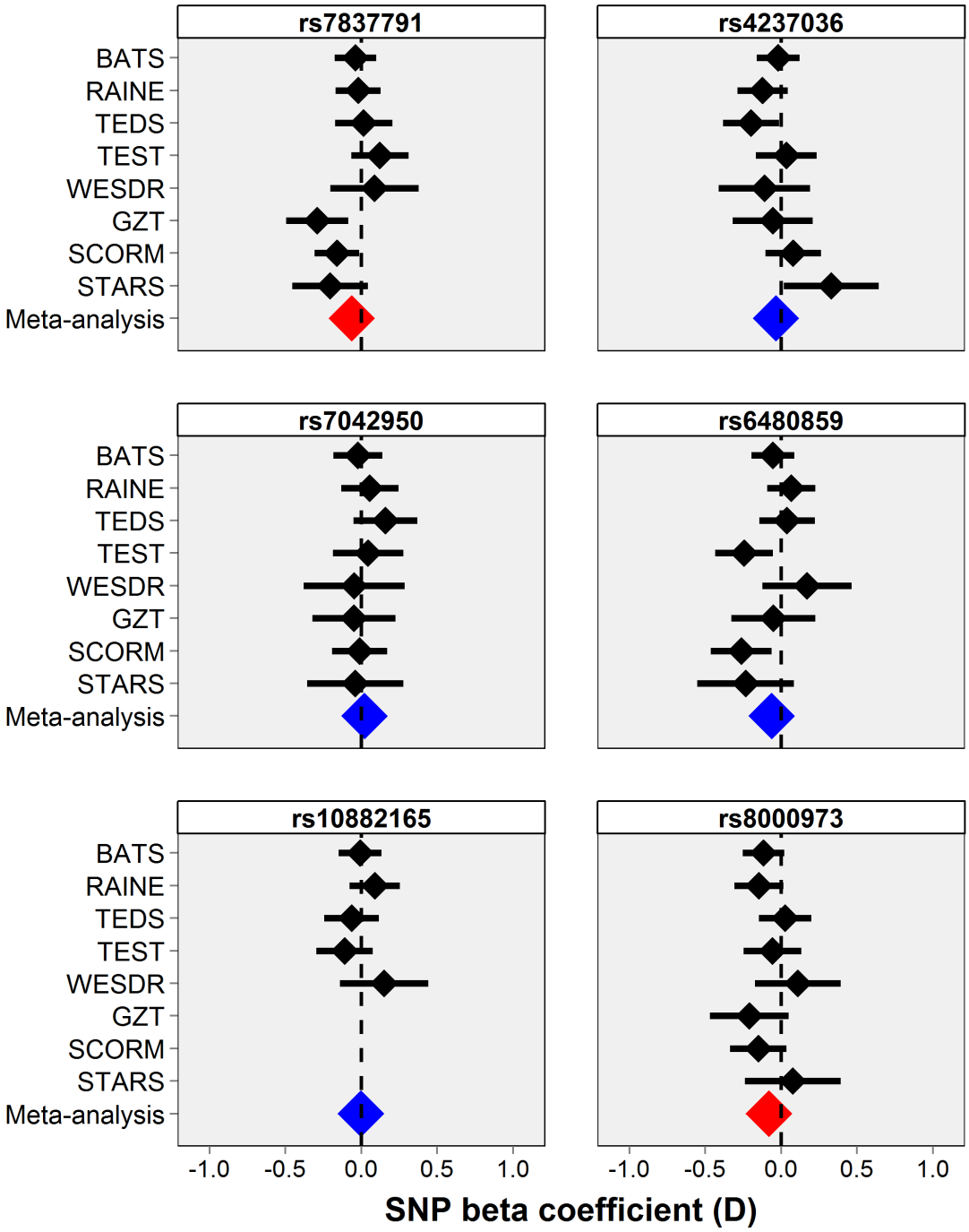


Figure S1 continued:

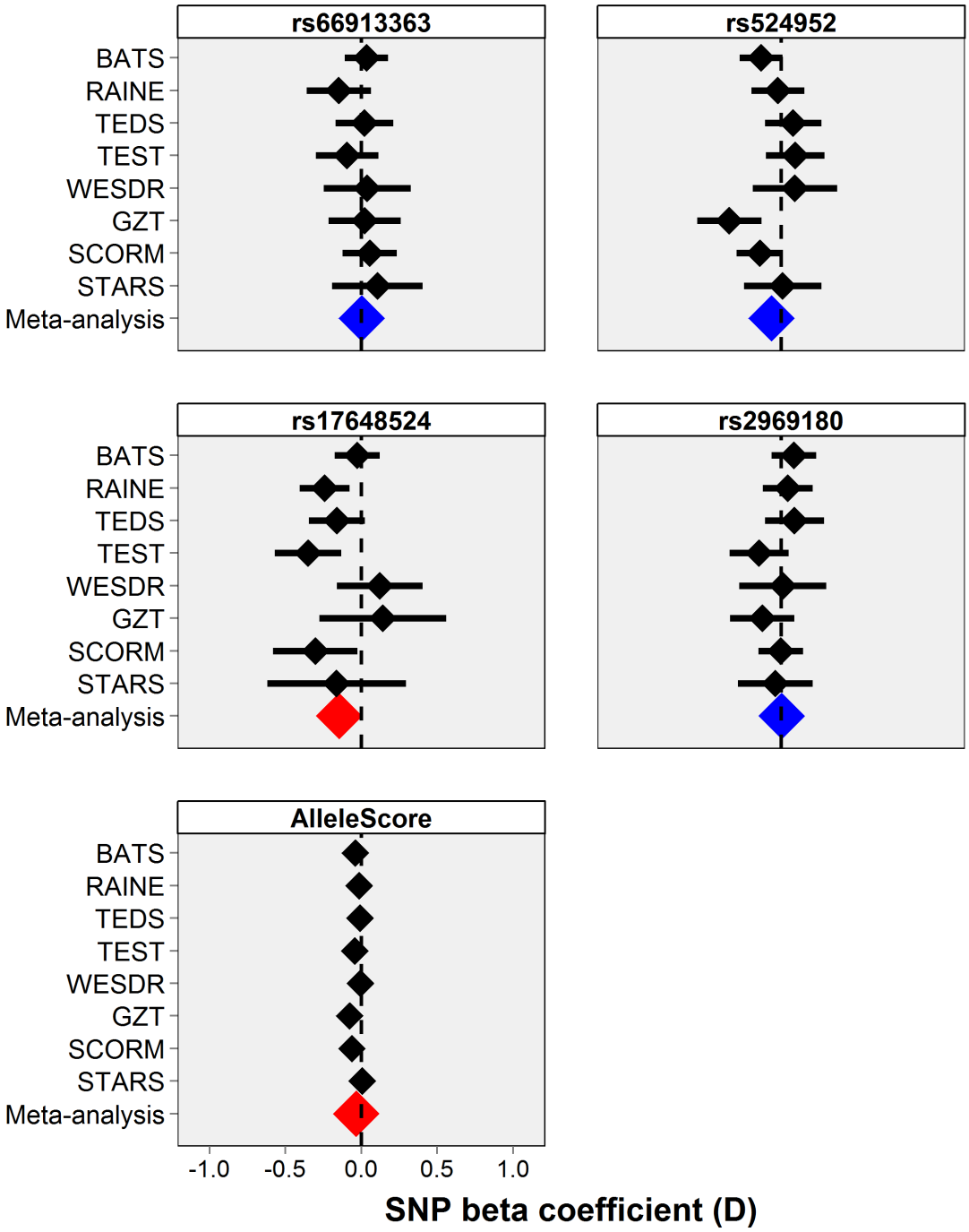
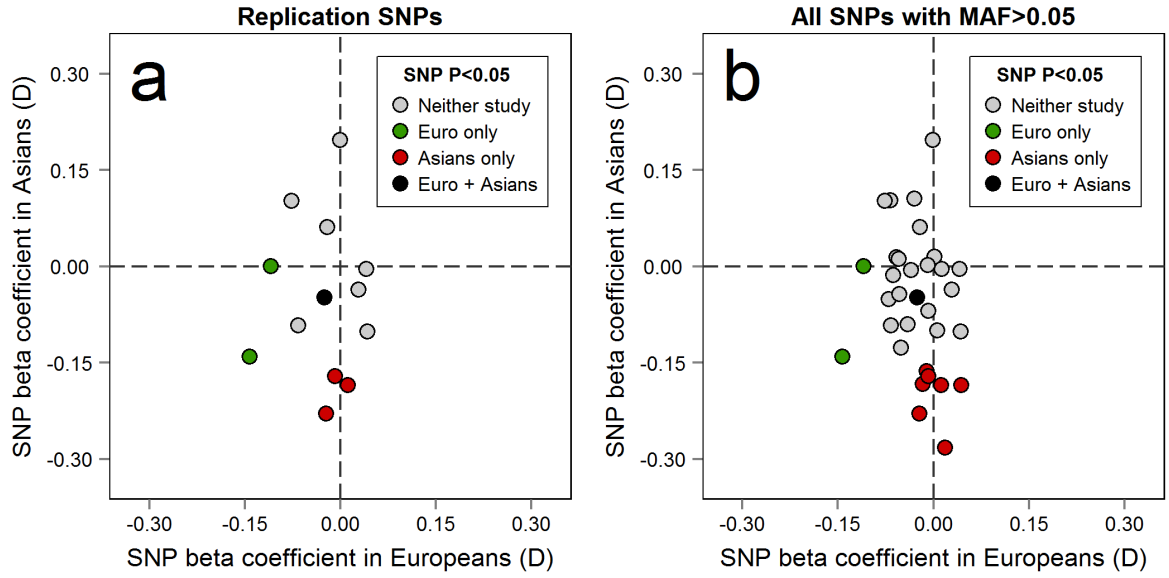
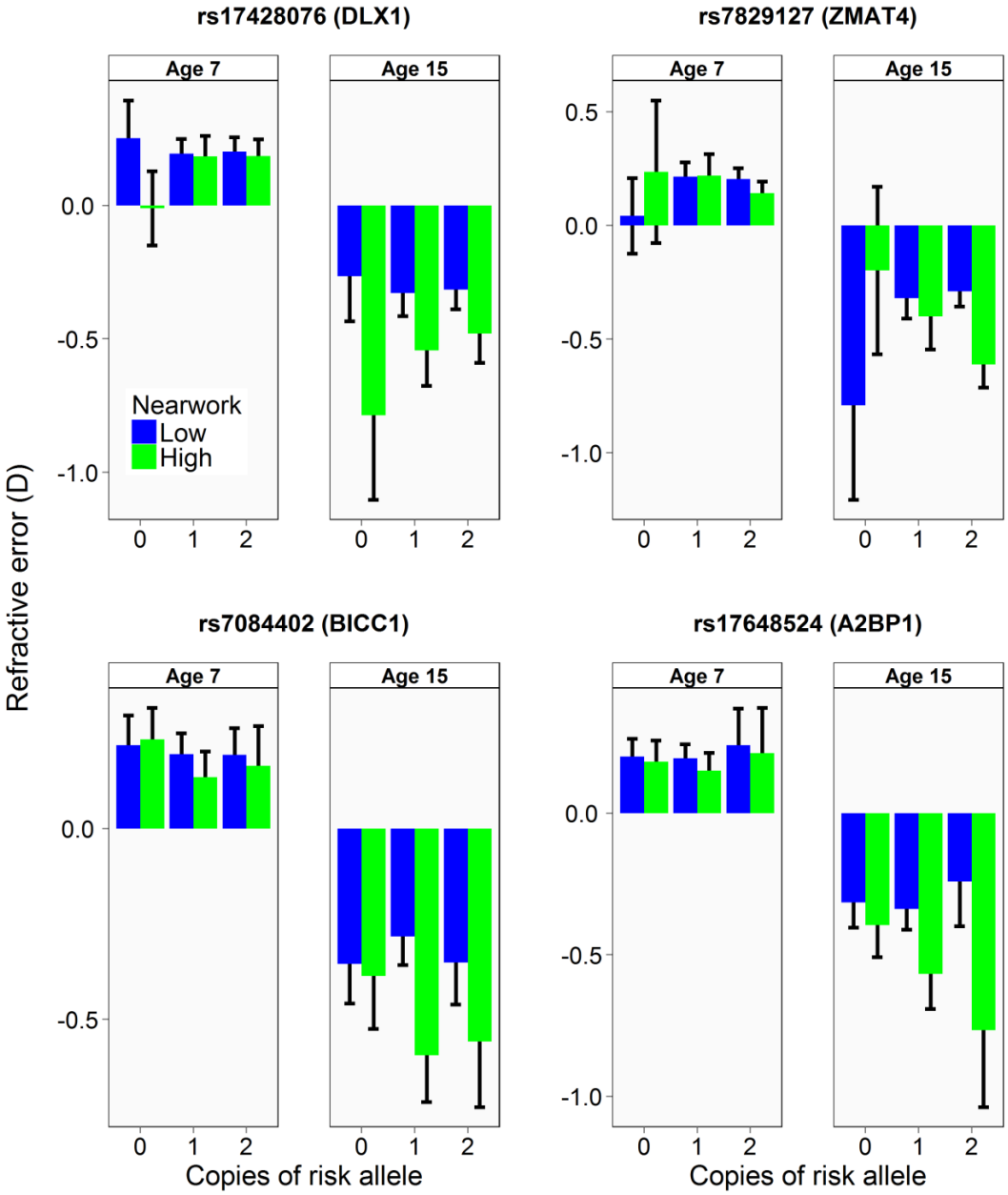


Figure S2. SNP effects in European and Asian meta-analysis samples. Beta coefficients from regression analysis (Dioptres per copy of the risk allele) for association with refractive error in meta-analyses of European and Asian individuals. Panel A: Results for the genetic risk score (black filled symbol) and 12 SNPs associated with refractive error in the ALSPAC longitudinal cohort (the set of "replication SNPs"). Panel B: All 31 SNPs with MAF>0.05 in both Asians and Europeans, plus the genetic risk score (black filled symbol).



Of the 12 SNPs with MAF>0.05 tested for replication in both ancestry groups, 9 had larger effects in Asians (P=0.07). Of 31 SNPs which had a MAF>0.05 in both ancestry groups, 20 had larger effects in Asians (P=0.07). The effect size of the 31 SNPs available for comparison was approximately 50% larger, on average, in Asian participants than in Europeans (-0.053 D, 95% C.I. -0.015 to -0.092 per copy of the risk allele in Asians versus -0.026 D, 95% C.I. -0.011 to -0.042 per copy of the risk allele in European participants) however this difference was within the range expected to occur by chance (P=0.21).

Figure S3. SNP x nearwork interactions at ages 7 and 15 in the ALSPAC discovery cohort. Refractive error at age 7.5 and age 15 was plotted for ALSPAC participants who were refracted at both ages (N=3,201) after grouping participants by SNP genotype and nearwork exposure. Graphs are presented for the 4 SNPs that showed 3-way SNP x nearwork x age-from-baseline interactions in the LMM analyses. Error bars show 95% CI.



Details of studies involved in meta-analysis (Appendix 9.4)

Chapter 6 | Discussion and Conclusions

6.1 Introduction

In this thesis I have explored the epidemiology and aetiology of myopia, using a number of different cohorts. Research findings were considered in the discussion section of included manuscripts but in this chapter I will further summarise the pertinent findings from each. I will then draw together my findings and discuss how they contribute to the current literature. Limitations will be discussed before I review the implications of my research and consider what direction my research and the field should go in the future.

6.2 Epidemiology of myopia

The prevalence of myopia is increasing, most dramatically in urban Asia but also in western populations (3, 50, 64). Whilst many small, single study or single country studies have been performed in Europe, no current, comprehensive estimates of the prevalence of refractive error had been performed prior to the work of the European Eye Epidemiology Consortium (E^3) and, particularly in the case of myopia, a current assessment of the burden of the condition was needed.

The E^3 consortium is a recently formed collaborative group of over 29 studies across 12 European countries inclusive of ophthalmic data on approximately 170,000 individuals (268). The aims of the consortium are "to promote and sustain collaboration and sharing of data and knowledge in the field ophthalmic epidemiology in Europe", with foci in a number of areas including the "estimation and projection of frequency and impact of visual outcomes in European populations". The use of previously collected datasets with refractive error provided a cost-efficient and rapid opportunity to produce prevalence estimates from a large overall sample.

Meta-analysed data from fifteen population-based, adult cohort and cross-sectional studies across Europe produced age-standardised prevalence estimates of 30.6% for myopia, 22.7% for hyperopia and 23.1% for astigmatism. A clear trend of higher myopia prevalence in younger participants was observed; between the ages of 25-45 years the myopia prevalence was approximately 40%, albeit with wide confidence intervals reflective of the relatively small number of participants falling within this age group in E^3. The highest myopia prevalence estimate was in the 25-29 years age group, at 47% (95% CI 41.8 – 52.5). In older populations the prevalence of myopia was lower; in the 70-74 years age group myopia prevalence was 13.9% (95% CI 11.9 - 15.9). The lower prevalence of myopia in older age groups could be attributed to two factors - the

known hypermetropic shift with aging (20) or, probably more likely, the observed cohort effect of rising myopia prevalence in younger generations. A hypermetropic shift with increasing age has been observed in emmetropes, myopes and hyperopes; in the Beaver Dam Eye Study the 10-year change in spherical equivalent was observed in approximately 2000 individuals aged 43-84 (22). The authors reported a 10-year change in spherical equivalent of +0.38D (95% CI 0.30 - 0.45), +0.24D (95% 0.16 - 0.31) and +0.25D (95% CI 0.19 - 0.32) in baseline emmetropes, myopes and hypermetropes respectively (when adjusted for age, gender, education, cataract grade, and diabetes). Extrapolating this, a low myope (eg. -1.5 D) aged ~40 years could feasible become emmetropic with a hypermetropic shift but only after a period of 40 years and providing they develop no lens-induced myopia. The estimates of myopia prevalence from E^3 are comparable but slightly higher than the 2004 Eye Diseases Prevalence Research group meta-analysis (6) and the US 1999-2004 NHANES (84).

The age-standardised prevalence of high myopia in E^3 was 2.7%, with the greatest levels of high myopia in the youngest participants (15-19 years old, myopia prevalence 5.9%). Different definitions of high myopia limit direct comparison but levels of high myopia in Europe appear to be similar to the US (84) and lower than urban Asia (220). Using 2010 European population data (269) and the myopia prevalence estimates from E^3, I estimated that there are 227 million people with myopia, of whom 20 million have high myopia, across Europe (270).

I identified clear evidence for increasing myopia prevalence in the latter part of the 20th century in Europe in the E^3 dataset. There was a 6% rise in age-standardised myopia prevalence rates between those born before 1940 compared to those born after, and at some ages the myopia prevalence was almost double for those born more recently - in 60-64 year-olds the myopia prevalence for those born in the 1920s was 15.2% (95% CI 12.7 - 17.7) whereas the prevalence for those born in the 1950s was 29.7% (95% CI 18.9 - 40.4). My publication describing these findings has provided the first evidence that myopia is becoming more common across Western and Northern Europe (270). The E^3 dataset provided an excellent opportunity to explore this issue as data on individuals of similar ages but with different years of birth were available – this meant the changing trend of age-specific myopia prevalence against decade of birth could be examined.

The next important question to examine was why it is becoming more common? Education is a well established risk factor for myopia and I like others found that those going onto higher education were much more likely to have myopia; the age-standardised myopia prevalence in those completing a primary, secondary and higher education was 25%, 29% and 37% respectively in E[3]. Age-specific myopia prevalence estimates revealed even larger differences between educational groups; myopia prevalence was approximately double for those going onto higher education compared to those with only primary education in certain age groups, for example in 50-54 year olds the myopia prevalence was ~24% in those completing a primary education and ~43% in those going onto higher education. I therefore hypothesised that this highly influential association must be driving increasing rates of myopia across Europe. However, despite the fact that rates of education have risen across Europe over the 20th Century, higher rates of myopia in younger generations were observed even within education strata. Those born more recently and completing higher education had approximately four times the 'baseline risk'. Therefore attainment of higher education alone cannot explain rising myopia rates in Europe, but we cannot exclude the effect of changing educational styles and practices.

In TwinsUK, an adult twin cohort predominantly female and largely aged 40-60 years, the mean age of myopia onset was 18 years. Almost half of those with myopia starting wearing glasses after the age of 17 years. This is comparable to the finding of another UK study, the 1958 British Birth Cohort, who also identified a significant amount of adult onset myopia (30), and a study from Argentina of adults born approximately in the 1960s who had a median age of myopia onset of 20 years (271). The influence of higher education and certain occupations, involving intense near work, may be influential in later onset myopia or myopia progression.

When I compare the age of myopia onset in comparable UK twin studies with a generational difference it appears the age of myopia onset is becoming younger; in the TEDS study the mean age of myopia onset was 10 years (SD 3.79). On re-examination of the TwinsUK cohort, in those who developed myopia before the age of nineteen the mean age of myopia onset was 12 years (SD 4.00). The suggestion that the age of myopia onset is becoming younger is reflected in the comparable age of myopia onset between TEDS and studies in other countries; for example 10.9 years in a Finnish cohort of myopic school children and 8.8 years in the SCORM study (13, 14). However, it should be noted these are paediatric studies and as such those with adult-onset

myopia are not captured in this estimate of age of onset and as the cohort ages the mean age of myopia onset may be higher. I can find no estimate of the age of myopia onset in a current, young adult cohort. In the 23andme study the mean age of myopia onset in their cohort of approximately 26,000 was 13.6 years (SE 5.8), but this cohort had a mean age of 48 years (SE 15.5) around 2010-2012 and there was no sub-analysis of the age of myopia onset in their younger participants (266).

The age of myopia onset explained only 15% of refractive error variance in TwinsUK and 4% in TEDS. There is a moderate correlation between the age of wearing glasses and final severity of myopia (r=0.39 in TwinsUK, and 0.42 in TEDS). The use of this measure as a proxy for measured spherical equivalent is discussed in chapter 5.3. I identified a greater number of significant associations when spherical equivalent was used as the outcome variable rather than age of spectacle wear, perhaps not unsurprisingly given that the genetic variants examined were initially identified using spherical equivalent as the outcome variable. Interestingly however two variants showed a stronger association with age of spectacle wear as the outcome variable raising the question if certain genetic variants are more associated with earlier onset myopia.

6.3 Environmental aetiology of myopia

The life course epidemiology approach on the TEDS cohort replicated known associations for myopia and also identified novel associations. Significant and suggestive associations with myopia were seen across childhood – most prominently for maternal education levels and cognitive variables in the twins. Ultimately only a small amount of the variance in myopia was explained by the 'candidate risk factors' examined, however twin modeling on the same dataset suggested that genetic factors far outweigh environmental effects in trait variance.

Early life factors associated with higher odds of myopia included maternal education prior to the birth of her child (OR 1.33) and a summer birth (OR 1.93). A summer birth, previously associated with myopia (235, 236), has been linked to higher light levels in the postnatal period. In TEDS there was no association with photoperiod (daylight hours) at birth. I believe the association between season of birth and myopia is reflective of a younger age at starting school for those born in the summer months; therefore implying that those who enter the education system younger, which we know is a highly 'myopigenic' environment, are at greater risk of developing myopia.

This hypothesis requires replication. A novel association between fertility treatment and reduced odds of myopia was identified (OR 0.63). This finding went against expectation but one possible explanation is that infants born following fertility treatment tend to have a shorter gestation period, lower birth weight and in some studies, but not all, have developmental delay and reduced cognitive scores (272-274).

In childhood and adolescence many factors were associated in univariable analyses but not retained in adjusted models. As discussed later, this may reflect lack of statistical power to detect associations in adjusted models or poor measures of the phenotype of interest. The only robust variable was time spent playing computer games, which was associated with a small but significant increased odd of myopia (OR 1.03). It is important however to consider the units of this odds ratio for myopia - number of hours spent playing computer games per week. Therefore the mean number of hours spent per week on computer games (5.3 hours) would mean the individual is 15% more likely to be more myopic. At the highest end of the spectrum three children reported spending 70 hours per week on computer games and this would make the child 210% more likely to be myopic; therefore this association can have a large effect on myopia risk. Whilst it is 'in vogue' to direct the blame for the 'myopia boom' at computers, iphones and tablets, it should be noted that the association in TEDS reflects the time spent playing video game consoles, likely via a TV, approximately eight years ago. As such, this is an intermediate viewing distance and, as suggested in the paper, may actually reflect long periods 'gaming' and spending less time outdoors. It may also be mediated by something in the broader personality of those who choose to play video game consoles compared to other activities. An association between certain personality types and myopia has previously been proposed with one study identifying an association between myopia and 'Openness' from the International Personality Item Pool inventory (242).

Over the life course suggestive associations at multiple age points were identified between myopia and cognitive variables, in particular verbal cognition. Whilst intelligence has been consistently associated with myopia (2, 148), the decomposition of different cognitive variables and the identification that verbal cognition is more correlated is novel.

In the EUREYE study elements of time outdoors, a well-replicated protective factor for myopia (185, 187), were investigated; namely a measure of sunlight (UVB) and both

serum vitamin D and genetic polymorphisms in vitamin D metabolism genes. In adjusted analyses lifetime UVB exposure was associated with a reduced odds of myopia (p=0.03), and the effect sizes were greatest in younger ages (age 14-19 years, OR 0.81, 95% CI 0.71-0.92). Lifetime exposure was measured using detailed, recall of sunlight exposure during adult life and did not include any measures before the age of fourteen. Given that myopia generally develops in childhood and that recall data is prone to error, the findings of this study are all the more remarkable. In common with the E^3 study, those going onto higher education, in the highest tertile of years of education, had twice the OR of myopia compared to baseline (OR 2.08). In this study, the effect size and significance of the association between myopia and UVB remained similar when adjusted for years in education. This suggests that the increased rates of myopia in those going onto higher education are not wholly mediated by a lack of exposure to sunlight.

I identified no association between serum vitamin D levels and myopia in the EUREYE study. Whilst there may be limitations in how well serum vitamin D in later life correlates with vitamin D in childhood, it was useful to replicate the work of others in finding no association (213). I also explored the association between genes for vitamin D metabolism and myopia, previously implicated as mediating the protective effect of sunlight (275), and found no association, without any limitation due to age of measurement given the stability of genetic variants.

6.4 Genetic aetiology of myopia

Refractive error was highly heritable in the TEDS cohort (86%), indicating that genetic factors explain the majority of refractive error variance. Twin studies are known to provide an upper estimate of the heritability of traits and the estimate obtained from TEDS was very similar to that obtained from other twin cohorts, including TwinsUK where the heritability estimate for right eyes was also 86% (252). Whilst I performed a GWAS in TEDS, the small sample size limited power to detect genome-wide significant variants. I was hoping to explore how well genetic variants from GWAS for refractive error in adults are replicated in children, however my finding of just two variants replicating (*A2BP1* and *PCC*) must be considered in light of the fact that I failed to replicate a highly frequent loci in Europeans (*GJD2*) that one would have expected to replicate.

The well-replicated association between myopia and intelligence has often been proposed as being due to an underlying shared genetic inheritance. The rather simple notion of 'big eyes, big brains' due a shared genetic risk has not been previously tested but the TEDS cohort, with data on both phenotypes, provided an excellent opportunity to explore this hypothesis. In TEDS the degree of phenotypic correlation, albeit relatively small (r=0.12), was found to be predominantly due to genetic factors (78%) in statistically significant estimates from a bivariate twin model. Then, using genotype data on the twins and previously published genetic variants associated with both traits, the proportion of variance explained by reciprocal trait—associated genetic variants was examined. In the case of refractive error, genome-wide polygenic scores for genetic variants associated with intelligence explained approximately 1% of variance, and comparably the proportion of intelligence variance explained by myopia-associated genetic variants was ~0.4%. Whilst the phenotypic correlation and proportion of trait variance are undoubtedly small, the findings of shared genetic variants and genetic correlation between the two traits appears robust.

The finding of shared genetic factors for myopia and education has previously been reported, albeit with insignificant path estimates in the twin modelling analysis (116). Polygenic risk scores based on genes for educational attainment explain 0.25% of refractive error variance (135). We identify a greater proportion of variance explained by intelligence (up to 1%), a phenotype we argue is 'purer' than education as the latter incorporates many different cognitive, social and demographic factors. Whilst a single gene for myopia and intelligence is very unlikely, I find evidence to suggest that multiple genes of small, contributory effect sizes may ultimately have pleiotropic functions. Embryonically both the neuroretina and brain arise from the same tissue, therefore expression of potential pleiotropic genes during periods of childhood growth and development could be postulated.

Gene by environment analyses in combination with other childhood studies participating in CREAM suggested that the genetic variants for myopia from adult GWAS are also associated in children. A greater proportion of refractive error variance was explained by these SNPs in adolescence compared to earlier in childhood in the discovery cohort (2.3% vs. 0.6%). Six genetic variants were associated with childhood myopia in European and Asian populations in replication samples. No significant interaction between a genetic risk score, comprising of 39 genetic variants identified for myopia in adult GWAS, and time outdoors was identified. In the discovery cohort

(ALSPAC) an interaction between time spent on near work activities and the aforementioned genetic risk score was identified for five genetic variants with nominal significance. Only one genetic variant (rs7829127, *ZMAT4*) survived correction for multiple testing. Replication was examined in four cohorts, including TEDS - none of the SNPs showed a significant interaction with near work. The heterogeneity in measures of time outdoors and near work may have confounded this work, and almost certainly greater power is required to gain significant results. Clearly further work in this area with larger samples and consistency in environmental measures is required.

6.5 Contributions to the literature and implications

The epidemiological work in this thesis contributes significantly to what is known about the burden of myopia, and other types of refractive error in Europe. No previous estimates exist with the same volume of participants or representation by various European countries. The current burden of myopia and the evidence that the prevalence is rising is key to the future planning of ophthalmic healthcare services; the work received much media interest (276, 277) but was also reported by agencies such as the International Agency for the Prevention of Blindness (278). It is relevant as many of those with myopia will be at risk of visual impairment in later life (35) and this has economic implications – potentially limiting employment of older working-age populations and independent living of the elderly. Interestingly, although TEDS is not truly population representative, the estimate of 35% myopia prevalence in 18-year olds suggests that additional individuals will still develop myopia (up to half of individuals developed myopia after the age of 17 in TwinsUK), and a similar prevalence to that observed in E^3 (47% in 25-29 year-olds) or higher would be expected in the TEDS cohort by early adulthood.

The answer as to why myopia is becoming more common in Europe is not fully answered in my research. While it could be hypothesised that education styles or practices may be more to blame for rising myopia levels, more work is required to tease out what factors are associated with the observed cohort effect. For example, lifestyle trends in modern society could mean less daily time spent outside.

It appears the age of onset of myopia is becoming younger both internationally and in the UK - in TEDS the age of myopia onset is 2 years younger than that seen in the sub-analysis of the comparable but older TwinsUK cohort. The reasons for this are likely

related to the same factors that are driving the rising epidemic of myopia (as opposed to those explaining trait variance). The implications of this are less clear. We know that age of myopia onset gives a prediction of final refractive error and potentially has a role as a proxy for when refractive error is not available; it is likely that a lower age of myopia onset will be associated with more people having a higher degree of myopia in adulthood in the future.

I was hopeful factors from the very detailed study of early life in the TEDS cohort would reveal associations with myopia. Whilst a number of associations were identified, including maternal education, season of birth and fertility treatment, many of the measures from the twins in their important pre-school years showed little or no association with myopia. Suggestive and unifying associations over the life course included cognitive and educational variables – the suggestion that verbal intelligence, in particular, is associated with myopia risk over the life course raises the question to what degree educational systems or intelligence underlie the well-replicated association between myopia and higher education.

There is much interest in the protective effect of time outdoors, particularly as it is now being used therapeutically to reduce myopia (189, 190). The reasons why time outdoors is protective are not fully answered and this was something I was keen to investigate by exploiting a pre-existing dataset on lifetime sunlight exposure and refractive error in Europe (EUREYE). We contributed to this field by finding evidence that in those going onto higher education, a strong risk factor for myopia, the protective effect of sunlight was maintained. We additionally confirmed no evidence for vitamin D or genes in vitamin D metabolism mediating this protective effect, in accordance with some, but not all, previous research (213, 275, 279).

Another important inference from the analysis of TEDS was in the twin modeling. The heritability of myopia in TEDS was very high (86%). This suggests that genetic risk factors underlie the majority of trait variance and that the life course associations identified can explain the majority of the 'environmental' component. However, what must be considered is how much the 'environmental associations' have a genetic element. Perhaps the most relevant example of this is maternal education – it is likely that the highest educational achievement by the mother is in part genetically determined. It could also be that genetic factors influencing maternal education could be passed onto their child and subsequently influence their educational potential or

even have shared effects on their risk of myopia. Similarly, a personality that is associated with a tendency to play computer games could be driven by genetic factors, and these again could have a shared relationship with myopia.

The question of shared genetics is something I explored in the correlation between myopia and intelligence. I chose to study intelligence, as I believed it provided a 'purer' phenotype for examination than say education, which incorporates so many factors. Whilst the phenotypic correlation between myopia and intelligence is small, the majority of this appears to be explained by genetics. This was observed in both the twin model analyses and when genotype data was examined.

There is undoubtedly a very complicated interplay between genes and environment, and what are termed 'environmental' risk factors may indeed have a 'genetic' element. Despite this, in collaboration with CREAM, I examined if there is an interaction between genes for myopia and certain environmental associations, namely near work and time outdoors. Whilst this study proved difficult due to heterogeneity and power, evidence for a statistical interaction between myopia and near work was identified for some genetic variants. It is likely that there are many other examples of this in myopia that could be examined in the future.

6.6 Limitations

Important limitations to this work must be acknowledged, and have already been discussed in each chapter. Consistently one of the most important to consider is the difference between correlation/association and causation. In all of the environmental associations I was exploiting retrospectively collected data for its correlation with myopia. This was unavoidable given the timeframe of my PhD, however my findings must be interpreted taking this into account. Associating one latent variable with another provides no means of implying causation or indeed a direction of causation. In order to test causation one would need to expose matched individuals to the environmental factor of interest or no exposure, and then monitor their refractive error over a period of time. In reality large sample sizes and treatment/placebo groups must be used - this is feasible for 'safe' exposures such as more time outdoors but it becomes very much difficult for educational delivery.

The TEDS study was initiated approximately 18 years before I started the myopia study and as such the analyses I undertook using the TEDS dataset were with retrospectively

collected data. Questionnaires specifically designed to assess, for example, time outdoors and near work in childhood with respect to myopia were therefore not used. This means I was using imperfect measures for phenotypes of interest, which limits my findings.

The representativeness of the datasets I have studied have limitations. In the E^3 dataset findings are limited to Northern and Western Europe with better prevalence estimates obtained for middle to older aged participants. Limitations and heterogeneity in terms of selection bias, drop-out bias, self-report of educational level achieved, and measurement of refractive error should also be acknowledged. To accommodate for this all meta-analyses were performed using a random-effects model. The E^3 dataset largely comprises of nationally or locally recruited population-based studies, however like all epidemiological studies a bias of more 'health conscious' volunteers participating may have occurred.

The EUREYE study similarly consisted of multiple European study centres but with the advantage of standardised testing protocols between centres and a more even coverage of Western, Northern, Southern and Eastern Europe. The main limitation of the generalisability of this study was the age of study participants (>65 years old) and that measured serum vitamin D at this age might not reflect serum vitamin D in childhood or young adulthood. Additionally, the use of recall data on time spent outdoors over the life course may be prone to bias, although the high association found for the exposure during adolescence in this study is reassuring. Retrospective findings could differ from a prospective study, and generational differences may mean that young adults questioned today may report different periods of time spent outdoors making these retrospective results less representative in the current era.

Twin datasets have been found to be representative of the broader population (247), however there are certain differences between twins and singletons – these include a shorter gestation period, greater likelihood of a caesarean assisted birth, lower birth weight (in part related to the first factor), and, in younger twin cohorts, a higher rate of fertility assisted conceptions (273). Whilst the TEDS study was recruited on the basis of national register, unlike TwinsUK that is a volunteer registry potentially subject to the 'healthy-volunteer' bias, the individuals who maintain contact with the study and choose to participate may be in some way be different to those who have lost contact with the study. As discussed in Chapter 7.5, the educational achievement of both the

mothers of the twins and the twins themselves in TEDS is higher in responders compared to non-responders. It is perhaps not surprising that educated individuals take a greater interest in medical research but it is another limitation of generalisability, particularly when it comes to estimates of prevalence. Reassuringly, the rates of myopia in TEDS are comparable to other UK estimates of adolescent myopia (102, 110) but I acknowledge associations identified in life course epidemiological analyses could be population-specific. TwinsUK is a volunteer registry however twins register without prior knowledge of specific myopia studies. Refraction is a small part of a much wider range of phenotype tests, and as such there is unlikely to be recruitment bias towards myopia or its risk factors.

Estimates of heritability from twin models are subject to a number of limitations. Firstly they are specific to the population being studied, and as such should not be blindly applied to be present in other populations or different generations of the same population. However, in the case of myopia, there are comparative estimates of heritability (252, 255). Heritability estimates are also affected by the age of the study group when an age-related trait is studied – specifically the wider the age group in the study of an age-related trait, the greater the effect of age (which is shared between twins, the same for MZ and DZ, therefore becoming part of the 'C' estimate) and the lower the estimate of heritability. Likewise, if a very narrow age group is examined then the effects of age may be missed (small 'C' estimate) and a heritability estimate will be high. This has been observed in the TwinsUK sample; in the initial "small" sample of 506 pairs the heritability was 84-86% with no estimate for 'C' (252), but when the sample size increased to over 2,000 pairs heritability estimates were lower because of a 7% estimate for 'C' (179). Therefore twin studies provide an upper estimate of heritability and the relative importance of a shared home environment of, for example, spending time outside, may be missed. In the case of bivariate twin models a certain degree of phenotypic correlation is required in order for the decomposition of the variance to be performed. Whilst the phenotypic correlation between myopia and intelligence was small, when decomposed significant estimates for relative contributions of genetic and environmental factors was obtained.

All molecular genetic analyses in this study are limited to the contribution of common genetic effects, therefore the contribution of rare genes and epigenetics is not estimated. The TEDS study alone did not have significant power to identify common genetic effects, let alone rare genetic effects, which require even larger sample sizes. It

is likely that multiple additive genetic variants contribute to the distribution of quantitative "complex" traits like refractive error in a population, and that common, complex conditions are mainly influenced by common genetic variants - the common disease common variant hypothesis (CDCV). This hypothesis states that common polygenic diseases are the result of common genetic variation in the population, typically sign-posted or marked by SNPs, that influence susceptibility to disease (280). However, the GWAS era has proven that a large amount of heritability remains unexplained; in GWAS for refractive error the ~40 loci published to date for refractive error still explain less than 5% of variation (265) and this is similar to what has been observed in other phenotypes such as height and type 2 diabetes (281). In a comparable cohort (ALSPAC) the total contribution of common genetic effects to childhood refractive error variance, also known as SNP-based heritability, was estimated to be 28% (282). This would suggest that common genetic variants, regardless of whether they have been confirmed to be associated with refractive error, could only ever explain around 30% of the variation of refractive error. If one considers twin modelling and the estimation of up to 86% of variation due to genetic factors, one can conclude that common genetic variation (as currently examined by standard array techniques) only accounts for a proportion of the genetic factors. This concept of 'missing heritability' is commonplace for polygenic, complex diseases (281). The contribution of genetic effects above those captured in modern GWAS using genotyping of common genetic variants is significant and complicated, encompassing rare variants, structural variations, epigenetics and interactions between genes and environment (gene-gene interactions and gene-environment interactions).

6.7 Future directions

Following the work of this thesis, there are a number of areas in which I would like to focus my future research efforts and where I feel the direction of myopia research may go.

Firstly, the work I have started, establishing the burden of myopia and other refractive errors across Europe, could be built upon and remains a high priority. The concern is that rising levels of myopia will result in more people with myopia in the future and that rates of associated visual impairment may increase. Therefore, current estimates of the degree of visual impairment associated with myopia and high myopia in a large pan-European study could be established and compared to previous estimates of 34% in high myopes at the age of 85 from the single site of the Rotterdam Study (283).

Further collaborative work between studies in E^3 that have both refractive error and (best-corrected) visual acuity data on their participants would enable an estimate of how much visual impairment is associated with myopia. This would ideally be analysed in conjunction with data on whether the individual has any ocular disease, in particular myopic maculopathy or myopic choroidal neovascularisation. Early discussions regarding this type of analysis suggest that there is a significantly lower level of visual acuity in moderate to high myopes compared to emmetropes. In the EUREYE study it was noted that of the twelve older aged individuals with high myopia, ten had choroidal neovascularisation (Hogg R and Fletcher A, Personal Communication). Whilst this ocular disease may be part neovascular age-related macular degeneration, the proportion of high myopes who incur this complication appears high.

It would also be useful to project the future burden of myopia using the meta-analysis prevalence data. Initial efforts to perform this type of analysis are underway with collaborators; using similar methods as used in a 2016 paper estimating the global burden of myopia from 2000-2050 (112), we aim to gain estimates of the age-specific myopia prevalence and number of people affected in Western Europe through to 2050.

Secondly, the TEDS and other twin datasets provide an excellent opportunity to explore genetic correlation between traits using a bivariate twin model. This captures all shared 'genetic' risk, including common genetic variation, rare genetic variation, and epigenetics. Genetic correlation can also be examined using genotype data in the form of Polygenic Risk Scores, Genome-wide Complex Trait Analyses, and LD-score regression. I utilised all of these methods for my analysis of the genetic correlation between myopia and intelligence, although only the first two methods gave me significant results and are included in this thesis. This methodology would be interesting to apply to other associations – for example how much of the link between myopia and maternal education is genetically mediated? Or perhaps, is there a genetic correlation between myopia and behavioural phenotypes associated with a tendency to play computer games? Whilst many of the associations in my analyses over the life course were considered 'environmental' they actually may be in part genetically mediated.

There is current interest in genetic correlation between traits; this means testing a set of genetic variants for association with two traits and examining if the effects of the genetic variants on the two traits correlated. In one study comparing large GWAS of 42

traits, a total of 341 loci were identified to be associated with multiple traits and several of these loci were associated with multiple phenotypes; for example genetic variants associated with schizophrenia were also associated with risk of inflammatory bowel disease (284). The authors looked at myopia, referred to as near-sightedness, using the 23andMe dataset with myopia determined by questionnaire responses rather measured refractive error in a sample of 106,086 cases and 85,757 controls. Twenty genomic regions with a variant that influenced more than six phenotypes were identified, of which five included the near-sightedness phenotype; these included loci on chromosomes 2, 3, 4, 16 and 19 as shown in Table 6.1. The authors additionally developed a method to identify causal relationships between pairs of traits; essentially they did this by looking at asymmetry in effect sizes of associated variants and applying thresholds of likelihood for a causal relationship. They identified sixteen pairs of traits using a non-stringent relative likelihood threshold (greater than 20) for a causal relationship and this included education and myopia.

Chr	Start	Stop	Putative causal gene	Phenotypes	Notes
2	26894985	28598777	GCKR	Platelet count, breast size, LDL, Crohn's disease, fasting glucose, triglycerides, height, **near-sightedness**, total cholesterol	often led by nonsynonymous SNP rs1260326
16	27445755	29036613	none	Any allergies, BMI, tonsillectomy, asthma, Crohn's disease, rheumatoid arthritis, parkinson disease, **near-sightedness**, educational attainment	large LD block at 28-29Mb
5	100678360	103221356	SLC39A8	Any allergy, height, Crohn's disease, parkinson disease, schizophrenia, HDL, **near-sightedness**	often led by nonsynonymous SNP rs13107325
19	44744108	46102697	APOE	Alzheimer disease, LDL, waist-hip ratio, triglycerides, HDL, total cholesterol, **near-sightedness**	Covers APOE
3	139954597	141339097	None	Any allergy, male-pattern baldness, Crohn's disease, mean red blood cell volume, height, **near-sightedness**	LD block covers ACFL2, ZBTB38, RASA2

Table 6.1 Genomic regions influencing more than six phenotypes including myopia. Adapted from (284). The 'putative causal gene' is given only if there is a non-synonymous SNP or otherwise functionally well-characterised allele among the strongest associations in the region. Abbreviations: chr = chromosome, LDL = low-density lipoproteins, BMI = body mass index, HDL = high-density lipoproteins

The major assumption of these analyses is that genetic pleiotropy is occurring, with a shared genetic inheritance for both traits. However, there are a growing number of examples in the literature where identified genetic correlations are more difficult to explain – for example educational attainment is negatively genetically correlated with

body mass index (285). The degree of genotypic and phenotypic correlation between myopia, environmental associations, and other ocular diseases merits further study.

Clearly a greater understanding into the genetic architecture of myopia would be valuable and efforts to increase the number of identified genetic variants of myopia are being aided by the large consortiums sharing genetic data and refractive error, such as CREAM (265), and the use of proxies for refractive error in 23andMe (266). This could enable a greater proportion of trait variance to be explained, develop our functional understanding of how myopia develops and would also enable further examination of genetic correlation and gene by environmental interactions.

I identified a number of novel associations that require replication, namely fertility treatment and my postulation that a summer birth is linked to myopia due to the fact that children born in those months start school at a younger age. The latter hypothesis implies that those entering the education system younger, which we know is a highly 'myopigenic' environment, are at greater risk of developing myopia. In order to replicate this finding I could approach similar cohorts such as ALSPAC but to confirm my theory I would need to propose a collaboration with researchers in the southern hemisphere where seasons are the opposite of those in the northern hemispheres yet academic terms remain broadly the same.

Finally, the ability to target myopia treatments is a high priority. The work in this thesis and that of others contributes to our understanding of who is likely to develop myopia. This will be increasingly relevant as the number of people with myopia, and high myopia, rises in the future. At present we know that refractive error in younger life, and potentially other ocular biometric measures, and parental myopia are potentially the best predictors of future myopia (11). It also appears lifestyle factors, namely time outdoors, may have a greater effect on myopia onset rather than progression (164, 168, 186-191).

Precision medicine is the ability to individually tailor health care on the basis of a person's genes, lifestyle and environment (286). Personalised patient care can only become more feasible with improved understanding into the genetic architecture of a trait and large epidemiological studies enabling identification of lifestyle associations. Large scale initiatives in the UK (100,000 Genomes Project (287)) and the US (Precision Medicine Initiative (288)) are have been set up in attempt to capture this

information for rare diseases. Common, complex conditions such as myopia present a greater challenge as the number of genetic variants and lifestyle factors contributing to overall risk is vast (289). Models to predict risk can be evaluated by receiver operator curves (ROC), which are a plot of sensitivity against 1-specificity, and an estimation of the area under the curve (AUC or AUROC) or C statistic. The AUC statistic is the probability that a randomly selected individual with the disease has a higher score than a randomly selected healthy individual (289). The value of an AUC statistic is between 0.5 (worthless) to 1 (perfect), and a rough guide is 0.9-1.0 is indicative of an excellent predictive model, 0.80-0.90 is good and 0.70-0.80 is fair. In age related macular degeneration, where GWAS have been successful in identifying many genetic variants, predictive models incorporating genetic, demographic and environmental variables confer good predictive ability; AUC statistics of 0.831 (290), 0.87 (291) and up to 0.907 when macular features at baseline are incorporated (292) have been obtained.

In myopia predictive models have been tested in longitudinal studies (12, 51, 52) (11, 162, 231); AUC statistics achieved range from 0.82 - 0.93 using model parameters including ocular biometry, visual acuity, parental myopia and visual activity. In TEDS the AUC statistic in the study of life-course risk factors was 0.68, and this did not include ocular biometry and parental myopia, or genetic associations (no comparable number of genetic associations to AMD with such strong effects have been identified for myopia). Refinement and improvement of predictive models will be possible when a greater number of genetic polymorphic markers and early life factors can be incorporated, enabling personalised medicine to have an increasing clinical role in myopia.

Improved understanding of the genetic architecture of myopia has a further potential clinical role in the identification of pathways underlying myopia development – this could enable identification of new therapeutic options and also an improved understanding of therapeutic efficacy (pharmacogenomics). Genetic polymorphisms influencing drug response have been identified in a number of conditions, mostly within the field of oncology (293). Therefore, in the future perhaps genotypes that respond better to treatments such as atropine or lifestyle interventions might be identified, that could be used clinically to target therapy in myopia (294).

6.8 Conclusions

In this thesis I have explored current trends in myopia epidemiology, modern lifestyle associations with myopia risk, and sought to better understand the complex genetic architecture of myopia. I have confirmed that myopia is becoming more common in Europe, with a lower age of onset in more recent British cohorts compared to older ones. Education is strongly associated myopia risk in all the datasets I have examined. Time outdoors, even when estimated retrospectively overly the life course, is clearly associated with a reduced odds of myopia but the mechanism underlying this association appears not to be higher vitamin D levels. I have attempted to tease out what factors underlie the strong association between myopia and education as the latter encompasses many different elements; I have found evidence for a small but significant shared genetic risk between myopia and higher intelligence. Additional collaborative analyses have highlighted an interaction between near work and some genetic variants associated with myopia. The complex interplay between the increasing number of common genetic variants identified for myopia and our environment, both in terms of genetic pleiotropy and gene-environment interactions, remains very interesting and merits further work.

Clearly prediction of who is likely to develop myopia will be very useful clinically to indicate who should receive a therapeutic intervention for myopia. This will become increasingly relevant in paediatric ophthalmology in the UK. Predictive models would also enable assessment of a valid level of risk to permit the widespread use of interventional treatments that are associated with potential side-effects, for example atropine therapy (177). An improved understanding of the genetic architecture of myopia will enable better polygenic risk prediction but could also determine who may respond better to certain therapeutics – pharmacogenomics. As we begin to better understand what polymorphisms are associated with myopia, their functional effects in the eye and how they may be synergistically involved with other phenotypes or environmental associations, we can begin to better target myopia prevention and treatment.

Chapter 7 | Materials

7.1 Introduction

In this section I will detail how the data for the TEDS Myopia Study was collected as this was my primary cohort and I personally collected the ocular data. The other studied cohorts (E^3, EUREYE and CREAM) were secondary analyses on existing cohorts and so I was not personally involved in the design or collection of eye phenotypes. However, further detail regarding these studies, in addition to the included publications, is provided in the associated supplementary information in the appendix section.

7.2 The Twins Early Development Study

The Twins' Early Development Study (TEDS) is a large, unique longitudinal study of 15,000 British twins, enrolled at birth between 1994 and 1996 from national birth records in England and Wales. The twins have been studied from a neurodevelopmental perspective over the life course; to date more than 5,000 pairs remain actively participating (295) and at the start of my study were aged between 16 to 18 years old. TEDS has remained representative of the UK population at various stages of contact [Table 4.1].

TABLE 1

Representativeness of the TEDS Sample at First Contact, Early Childhood, Middle Childhood, and Adolescence

	Returned data (*N* families)	% Response rate	% White	% A-levels or higher	% Mother employed	% Father employed	% Female	% MZ
First contact	13,694	84.0%	91.7%	35.5%	43.1%	91.7%	50.1%	33.1%
Early childhood	10,150	69.3%	92.9%	38.1%	43.4%	92.3%	50.9%	33.5%
Middle childhood	8,819	59.1%	93.2%	39.8%	46.0%	92.9%	51.0%	35.0%
Adolescence	8,697	74.1%	92.8%	40.1%	46.4%	92.8%	51.7%	34.7%

Note: The equivalent UK population percentages for this generation are 93% white; 32% for A-levels or higher; 49% for mother employed; and 89% for father employed (Walker et al., 2001). Early childhood refers to families who provided any data when the twins were aged 2, 3, or 4 years. Middle childhood refers to families who provided any data when the twins were aged 7, 9, or 10 years. Adolescence refers to families who provided any data when the twins were aged 12, 14, or 16 years. A-levels are the national educational qualification taken at 18 years of age in the UK, and refer to parental educational qualifications. % female relates to the sex of the twins. MZ = monozygotic twins. These percentages for MZ twins match the expected distribution of twin births in the United Kingdom.

Table 7.1 TEDS demographic representation during the life course, reproduced from (295, 296)

TEDS has studied the development of language, cognition, academic abilities and behaviour from multivariate quantitative and molecular genetic perspectives. The twins' parents, the twins' teachers and the twins themselves have completed numerous assessments at multiple time points throughout their childhood, in the form of home visits from researchers, paper questionnaires, telephone assessments and web-based tests. TEDS has considerable experience of twin model-fitting techniques to investigate

genetic and environmental influences in multivariate traits; their Generalist Genes Hypothesis suggests a single set of genes has general effects across diverse learning abilities/disabilities (297, 298). GWAS of 4,000 twins (one twin per pair) have already been performed, and analyses for reading, maths and cognition have been published (299, 300).

7.3 Data Collection

Ethical approval was obtained to contact a sub-group of TEDS to participate in the myopia study (King's College London Psychiatry, Nursing and Midwifery Research Ethics Subcommittee PNM/11/12-140). This sample of 1,754 MZ and 3,466 DZ twin pairs was selected to include those more actively involved in TEDS, who continue to reside in the UK and in whom GWAS data was available. A brief questionnaire, together with study information, was sent to each twin with questions relating to the need for glasses and contact lenses, age of spectacle wear if applicable, the date and location of their most recent eye test, and a consent form for study participation (Appendix 9.1). This was signed by the parent if the twin was under the age of 16 years or by the twins themselves if the twin was 16 years or older, with a preference for parental signature in addition. A "health promotion" reminder that all children in full time education in the UK are eligible for a free eye test was included. Reminder letters were posted to all non-responders one month following the original mail out. This element of data collection was performed in collaboration with administrators at the TEDS office and their contact details were provided to allow twins to contact a familiar member of TEDS team with any questions.

Data was entered onto an access database by temporary administrative staff, volunteers and KW. Optician contact details were confirmed using a web search if incomplete. Postal questionnaires were sent to opticians, together with a copy of the consent form, with box entries for subjective refraction, visual acuity unaided and best-corrected, glasses or contact lenses prescribed, and presence of amblyopia or squint (Appendix 9.2). Contact details for KW were provided for any queries and a postal reminder was performed two months later. To increase response rate further, KW performed phone call reminders three months after initial mail-out.

The collection of refractive error data was commenced with the sending of questionnaires to the twins in September 2012 (n=5250); a 52% response was

achieved (n=2,706). Of those who responded, 84% reported attending a named optician for eye test and 45% reported wearing either glasses or contact lenses. Optician questionnaires were mailed in spring 2013 with subsequent reminder letters and finally reminder phone calls. Ultimately refractive error was available for 1996 twins, and 1991 twins with complete demographic information, namely age at examination.

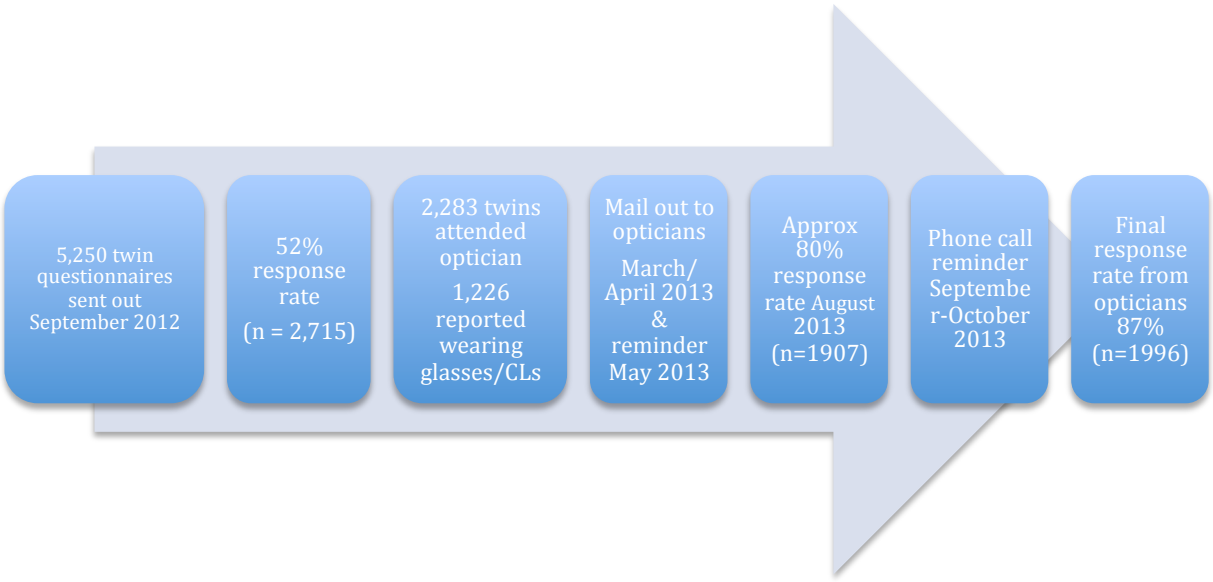


Figure 7.1 Progress of refractive error data collection

7.4 Refractive error classification

Refractive error data obtained enabled two outcome measures, the continuous quantitative trait of spherical equivalent (calculated in the standard manner using the formula *sphere + (cylinder/2)*) and dichotomous trait of presence/absence of refractive error as defined below. The mean of the two eyes was considered unless data was only available for one eye.

Definitions	
Myopia	**≤ -0.75 D**
Low Myopia	≤ -0.75 to -3.00 D
Moderate Myopia	≤ -3.00 to -6.00 D
High Myopia	≤ -6.00 D
Hypermetropia	**≥ + 1.00 D**
Low Hypermetropia	≥ + 1.00 to + 3.00 D
High Hypermetropia	≥ + 3.00 D
Astigmatism	**≥ 1.00 D in either eye**

Table 7.2 Refractive classification definitions

7.5 Assessment of representativeness of cohort

The responders and non-responders of the TEDS myopia sample were analysed for any significant differences that might bias results. Differences in gender, ethnicity, parental education, parental employment, and educational achievement were compared.

Participants of TEDS were reflective of the general population (see 7.2). Responders and non-responders of the TEDS myopia study were collectively very comparable to the full TEDS cohort. The differences between TEDS myopia cohort responders and non-responders are in Table 7.3 below; ethnicity, paternal employment, maternal, employment, gender and zygosity appear comparable for responders and non-responders in the myopia study. Responders and non-responders of the TEDS myopia study were collectively also very comparable to the full TEDS cohort. The only notable difference is a higher proportion of parental A-levels, especially in mothers, in both the full TEDS study and TEDS myopia study responders compared to the TEDS myopia study non-responders. When compared the 2011 census data it also appears the current UK population has a higher prevalence of non-white ethnicity and a significantly higher proportion of maternal employment compared to the TEDS cohort and previous UK data (296). Parental employment in the TEDS myopia study responders is comparable to UK census data, whilst the lower proportion of parental employment in non-responders appears less representative of the UK population.

Twin participants' academic achievements appear slightly higher in responders when compared to both non-responders and 2011 national statistics (301)

	TEDS adolescence contact (responders)	TEDS Myopia study (responders)	TEDS Myopia study (non-responders)	UK Census data 2011 (302)
n (%)	8,697 (74.1%)	2,715 (51.7%)	2,535 (48.3%)	
% White	92.8%	95.3%	94.0%	86%
% Parental A-levels	40.1%	Mother: 41.0% Father: 39.5%	Mother: 26.4% Father: 28.2%	39%
% Mother employed	46.4%	48.6%	46.1%	76.5% (303)
% Father employed	92.8%	95.1%	92.4%	89.5%(303)
% Female	51.7%	55%	52.4%	50.9%
% MZ	34.7%	34.0%	33.2%	-
% ≥5 A* to C GCSE passes	-	89.5%	83.8%	79.6% (301)

Table 7.3 Representativeness of TEDS Myopia Study responders

7.6 Distribution and prevalence of refractive error

Refractive error data is available for 1996 (87% of those who attended an optician). There was a slight female predominance of 58% (n=1,159) and 96% of the cohort were White European. The mean spherical equivalent was -0.35 D (SD 1.80) with a range from -10.13 to +10.50 D. The mean age at refraction was 16.3 years (SD 1.75). Amblyopia was reported in 5.4% and 4.3% had a documented squint. The distribution of refractive error displayed a typical leptokurtotic distribution, with a slight negative skew towards myopia [Figure 7.2]. Study participants were between 16 to 18 years old at the time of the myopia study initiation, however many reported their most recent eye test occurring some years prior. Therefore the age at refraction ranged from 5 to

18 years, with predominance of refractions between the ages of 15 to 18 years and a trend towards a more myopic prescription in these older participants [Figure 7.3].

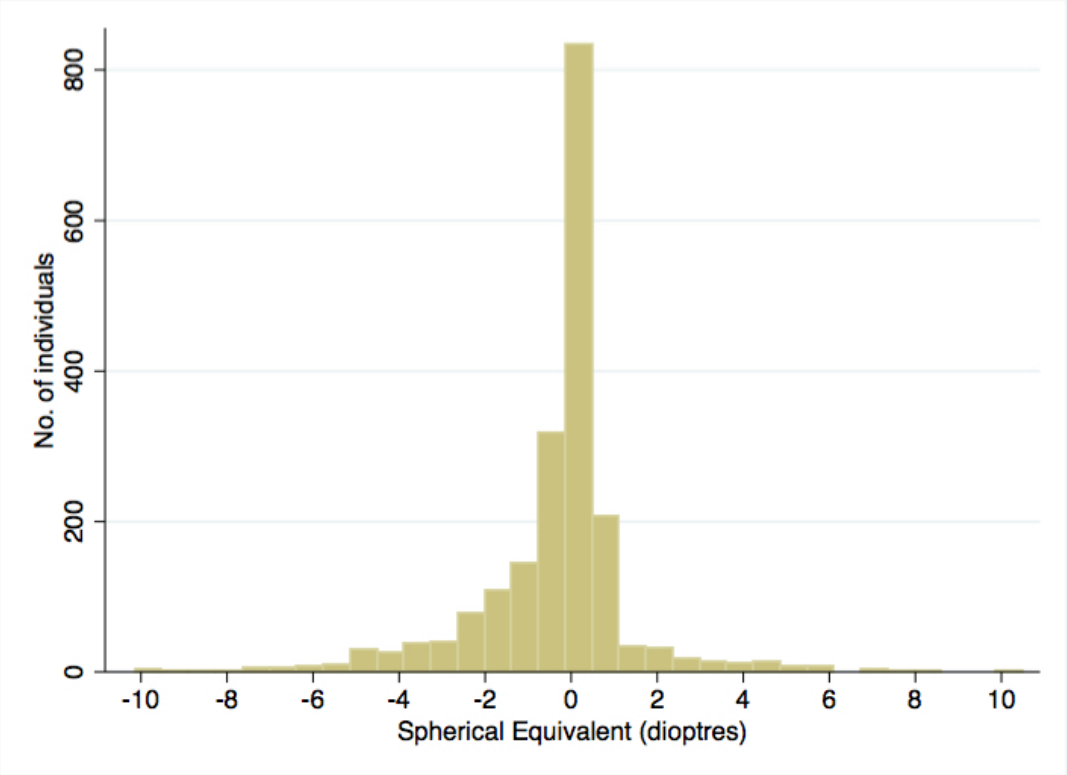


Figure 7.2 Histogram of spherical equivalent (n=1996)

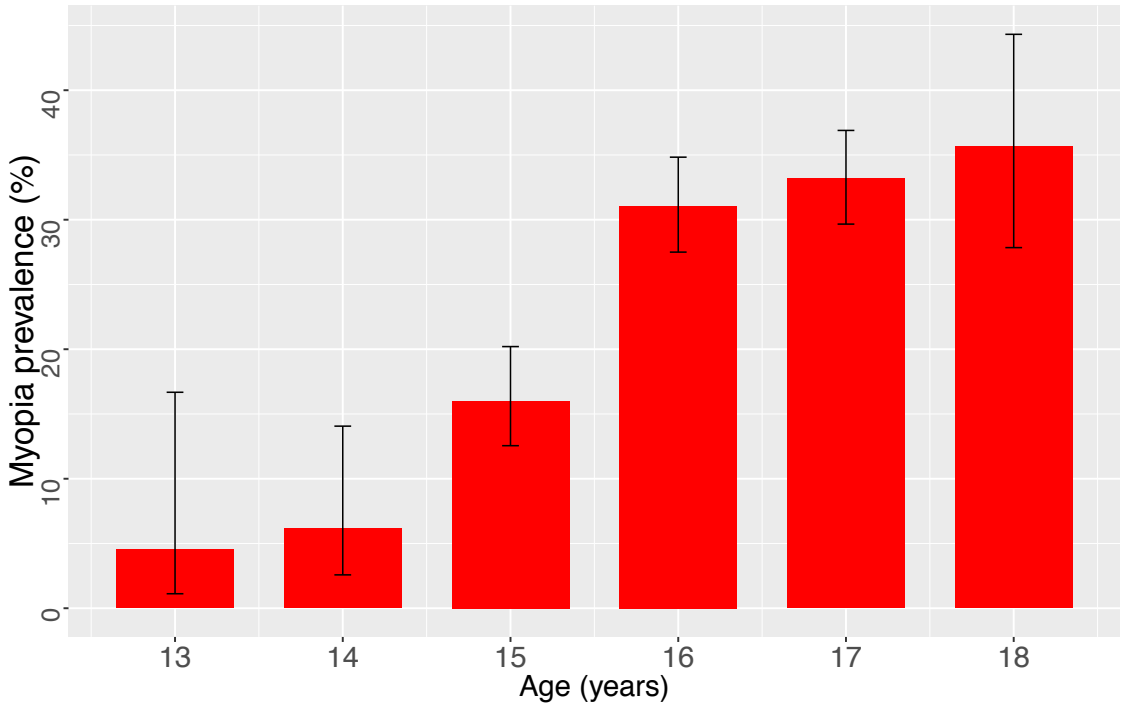
Figure 7.3 Distribution of refractive error by age

The sample prevalence of refractive error was observed for the total cohort and in two-year age groups, to examine the effect of age [Table 7.7]. The overall prevalence of myopia was 26.0% (95% CI 24.1-27.9), with high myopia in 1.2% (95% CI 0.8-1.7) of the subjects, and there was a significant trend with age. The overall prevalence of hypermetropia was lower at 8.0% (95% CI 6.9-9.3) with a higher proportion of higher-grade hypermetropia (3.1%), and a less marked variation with age. The prevalence of astigmatism was estimated at 12.7% (95% CI 11.3-14.2). The majority of the cohort was between 16-18 years old and so these age ranges provide the narrowest confidence intervals; myopia prevalence was 32.5% (95% CI 30.1-35.0) and hyperopia at 8.3% (95% CI 7.0-9.9) in this age group.

	Total		< 14 years old (n=162)		14 to <16 years (n=437)		≥ 16 years old (1397)	
	n	**% (95% CI)**	**n**	**% (95% CI)**	**n**	**% (95% CI)**	**n**	**% (95% CI)**
Myopia	518	26.0 (24.1-27.9)	2	1.2 (0.3-4.9)	62	14.2 (11.2-17.8)	454	32.5 (30.1-35.0)
Low	376	18.8 (17.2-20.6)	2	1.2 (0.3-4.9)	48	11.0 (8.4-14.3)	326	23.3 (21.2-25.6)
Moderate	119	6.0 (5.0-7.1)	0	0	13	3.0 (1.7-5.1)	106	7.6 (6.3-9.1)
High	23	1.2 (0.8-1.7)	0	0	1	0.2 (0.0-1.6)	22	1.6 (1.0-2.4)
Hyper-metropia	160	8.0 (6.9-9.3)	7	4.3 (2.1-8.9)	37	8.5 (6.2-11.5)	116	8.3 (7.0-9.9)
Low	98	4.9 (4.0-6.0)	7	4.3 (2.1-8.9)	24	5.5 (3.7-8.1)	67	4.8 (3.8-6.1)
High	62	3.1 (2.4-4.0)	0	0	13	3.0 (1.7-5.1)	49	3.5 (2.7-4.6)
Astigmatism	253	12.7 (11.3-14.2)	5	3.1 (1.3–7.3)	33	7.6 (5.4–10.4)	215	15.4 (13.6 -17.4)

Table 7.4 Prevalence of refractive error in TEDS Myopia Study

A marked increase in the prevalence of myopia was observed with a peak prevalence of 36% in those aged 18 years. As previously observed the prevalence of hyperopia was lower with less variation by age.



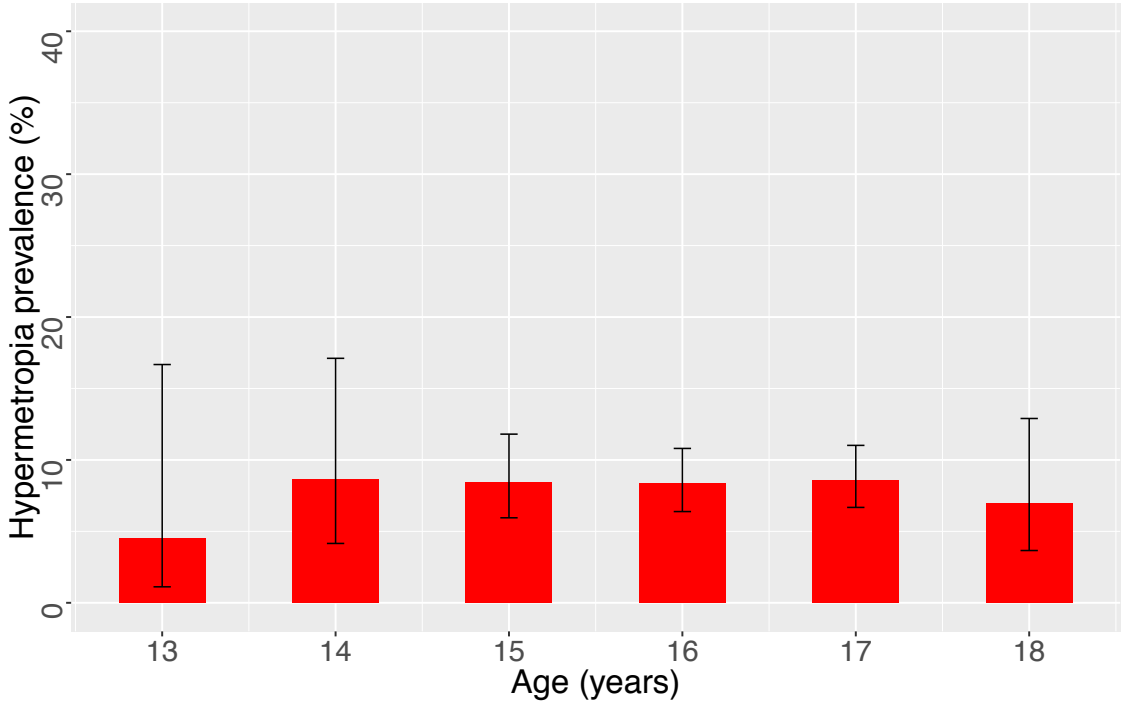


Figure 7.4 Prevalence of myopia and hypermetropia by age at refraction

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Appendix

8.1 TEDS Myopia Study - questionnaire to the twins

TEDS Research Centre
Dept. Box No. P083
FREEPOST LON7567
London SE5 8YZ

Freephone 0800 317029
Email: teds-project@kcl.ac.uk
Website: www.teds.ac.uk

TEDS Myopia Study: Eyesight Questions

<Twin 1 forename's> Eye Sight:

1. (a) Have you ever had an eyesight test? Yes □ No □
   (b) **If yes**, in what year was your last test? _____ (guess if not sure)
   (c) Please provide details of the optician (name and town is sufficient) at which you most recently had your eyes tested, **even if you do not wear glasses or contact lenses**

   Name: _____

   Address: _____

   _____

   _____

   Phone number: _____

2. (a) Have you ever worn
   (i) glasses? Yes □ No □
   (ii) contact lenses? Yes □ No □
   (b) **If yes**, at what age did you first start wearing glasses or contact lenses? _____ years

<Twin 2 forename's> Eye Sight:

1. (a) Have you ever had an eyesight test? Yes □ No □
   (b) **If yes**, in what year was your last test? _____ (guess if not sure)
   (c) Please provide details of the optician (name and town is sufficient) at which you most recently had your eyes tested, **even if you do not wear glasses or contact lenses**

   Name: _____

   Address: _____

   _____

   _____

   Phone number: _____

2. (a) Have you ever worn
   (i) glasses? Yes □ No □
   (ii) contact lenses? Yes □ No □
   (b) **If yes**, at what age did you first start wearing glasses or contact lenses? _____ years

<FamilyID>

8.2 TEDS Myopia Study - questionnaires to optometrists

TEDS Research Centre
Dept. Box No. P083
FREEPOST LON7567
London SE5 8YZ
Freephone 0800 317029
Email: teds-project@kcl.ac.uk
Website: www.teds.ac.uk

September 2012

Dear Name and Address of Optician,

TEDS Myopia Study
REC Reference Number: PNM/11/12-140

RE: Name, DOB and address of twins

We have received consent from _____ & _____ to take part in the TEDS Myopia Study, and are now writing to you in order to collect data on their most recent refraction. The aim of this study is to examine the complex influence of genes and environment on a person's risk of developing myopia. We hope this research will provide new information on why myopia develops and why the incidence is increasing. This study is being conducted by Dr Katie Williams, working with Professor Robert Plomin of the TEDS study group and Professor Chris Hammond of the Department of Ophthalmology at King's College London.

The Twins Early Development Study is one of the foremost ongoing twin studies in the world (www.teds.ac.uk). With the help of over 15,000 families, TEDS researchers are exploring how we develop through childhood and adolescence. The study is providing insights into how both nature (the genetic material and characteristics we inherit from our parents) and nurture (our environment) contribute to individual development in a range of areas such as cognition, learning abilities and behaviour.

_____ & _____ have been part of the TEDS study for over 15 years and have now kindly agreed to take part in this new phase. They have provided their consent (attached) for you to release details regarding their eye health, refraction and need for glasses or contact lenses. In order for us to collect accurate data we would be very grateful if you could return the attached questionnaire using the prepaid envelope.

All data collated will be kept strictly confidential and stored on a secure server. This is a King's College archive with authorized access to the anonymized data given only to TEDS researchers (and collaborators) by the data manager, at the discretion of the TEDS Director (Robert Plomin). A copy of the study report on completion will be available.

Thank you for your support of the Twins Early Development Study.

Yours sincerely,

Professor Robert Plomin

Family ID: <FamilyID>

TEDS Myopia Study Optician Questionnaire

Name: <twin forename + surname>

1. **What is the most recent refraction?**

   Date: _ _/ _ _/ _ _ _ _

	Sphere		**Cylinder**			**Unaided Visual Acuity**	**Best Corrected Visual Acuity**
	+/-	Power (to nearest 0.25D)	+/-	Power (to nearest 0.25D)	Axis (0 – 180 degrees)		
Right Eye							
Left Eye							

2. **Was the individual prescribed glasses or contact lenses?**

   Glasses yes □ no □

   Contact Lenses yes □ no □

3. **Does the individual have any of the following ocular comorbidities:**

   Ambylopia yes □ no □

   Squint yes □ no □

Name: <twin forename + surname>

1. **What is the most recent refraction?**

   Date: _ _/ _ _/ _ _ _ _

	Sphere		**Cylinder**			**Unaided Visual Acuity**	**Best Corrected Visual Acuity**
	+/-	Power (to nearest 0.25D)	+/-	Power (to nearest 0.25D)	Axis (0 – 180 degrees)		
Right Eye							
Left Eye							

PLEASE TURN OVER

Family ID: <FamilyID>

2. **Was the individual prescribed glasses or contact lenses?**

   Glasses yes ☐ no ☐

   Contact Lenses yes ☐ no ☐

3. **Does the individual have any of the following ocular comorbidities:**

   Ambylopia yes ☐ no ☐

   Squint yes ☐ no ☐

<Name or branch of optician>

Signature:

Date:

8.3 TEDS Myopia Study Ethical Approval

Katie Williams
Department of Twin Research and Genetic Epidemiology
King's College London
St Thomas' Hospital Campus
1st Floor South Wing Block 4
Westminster Bridge Road
London SE1 7EH

17 August 2012

Dear Katie

PNM/11/12-140 Cognitive, behavioural, environmental and genetic associations of myopia in the Twins Early Development Study.

Review Outcome: Full Approval

Thank you for sending in the amendments/clarifications requested to the above project. I am pleased to inform you that these meet the requirements of the PNM RESC and therefore that full approval is now granted with the following provisos:

1. Myopia Invitation letter: Please state that the study has been approved by King's College London Psychiatry, Nursing and Midwifery Research Ethics Subcommittee, followed by the reference number.

2. Information Sheet: In the first paragraph please include the full title of the Subcommittee 'King's College London Psychiatry, Nursing and Midwifery Research Ethics Subcommittee'.

3. Consent Form:
    I. Please note that participants can withdraw from a study at any time, however you can specify a period for the withdrawal of their data. As such, please add a bullet point and tick box and include the following sentence: I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and withdraw from it immediately without giving any reason. Furthermore, I understand that

I will be able to withdraw my data during the study period September 2012 to August 2013. This should be the first bullet point on the Consent Form.

II. Add a bullet point and tick box stating 'I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the Data Protection Act 1998'.

Note that you do not need to submit a response to the above provisos, however it is a condition of the approval granted by the PNM RESC that the provisos are carried out prior to the study commencing. If the provisos are not adhered to, the approval granted by the PNM RESC would no longer be valid. Should you have any queries on this please do not hesitate to contact the Research Ethics Office.

Please ensure that you follow all relevant guidance as laid out in the King's College London Guidelines on Good Practice in Academic Research (http://www.kcl.ac.uk/college/policyzone/index.php?id=247).

For your information ethical approval is granted until **17 August 2015**. If you need approval beyond this point you will need to apply for an extension to approval at least two weeks prior to this explaining why the extension is needed, (please note however that a full re-application will not be necessary unless the protocol has changed). You should also note that if your approval is for one year, you will not be sent a reminder when it is due to lapse.

Ethical approval is required to cover the duration of the research study, up to the conclusion of the research. The conclusion of the research is defined as the final date or event detailed in the study description section of your approved application form (usually the end of data collection when all work with human participants will have been completed), not the completion of data analysis or publication of the results. For projects that only involve the further analysis of pre-existing data, approval must cover any period during which the researcher will be accessing or evaluating individual sensitive and/or un-anonymised records. Note that after the point at which ethical approval for your study is no longer required due to the study being complete (as per the above definitions), you will still need to ensure all research data/records management and storage procedures agreed to as part of your application are adhered to and carried out accordingly.

If you do not start the project within three months of this letter please contact the Research

Ethics Office.

Should you wish to make a modification to the project or request an extension to approval you will need approval for this and should follow the guidance relating to modifying approved applications:
http://www.kcl.ac.uk/innovation/research/support/ethics/applications/modifications.aspx
The circumstances where modification requests are required include the addition/removal of participant groups, additions/removal/changes to research methods, asking for additional data from participants, extensions to the ethical approval period. Any proposed modifications should only be carried out once full approval for the modification request has been granted.

Any unforeseen ethical problems arising during the course of the project should be reported to the approving committee/panel. In the event of an untoward event or an adverse reaction a full report must be made to the Chair of the approving committee/review panel within one week of the incident.

Please would you also note that we may, for the purposes of audit, contact you from time to time to ascertain the status of your research.

If you have any query about any aspect of this ethical approval, please contact your panel/committee administrator in the first instance (http://www.kcl.ac.uk/innovation/research/support/ethics/contact.aspx). We wish you every success with this work.

With best wishes

Yours sincerely

Catherine Fieulleteau
Senior Research Ethics Officer

Cc: Professor Robert Plomin

Professor Chris Hammond

8.4 Supplementary Information for cohorts contributing to the presented E³ analyses

The Alienor study

The Alienor (Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes) Study is a population-based prospective study aiming at assessing the associations of age-related eye diseases (age-related maculopathy, glaucoma, cataract, dry eye syndrome) with nutritional factors (in particular antioxidants, macular pigment and fatty acids), determined from plasma measurements and estimation of dietary intakes. It also takes into account other major determinants of eye diseases, including gene polymorphisms, environmental factors and vascular factors. The methods of this study have been published elsewhere (304).

Subjects of the Alienor Study were recruited from an on-going population-based study on the vascular risk factors for dementia, the Three-City (3C) Study (305). The 3C Study included 9,294 subjects aged 65 years or more from three French Cities (Bordeaux, Dijon and Montpellier), among whom 2,104 were recruited in Bordeaux. They were initially recruited in 1999-2001 and followed-up about every two years since. The Alienor Study consists of eye examinations, which are proposed to all participants of the 3C cohort in Bordeaux since the third follow-up (2006-2008). Among the 1,450 participants re-examined between October 2006 and May 2008, 963 (66.4%) participated in the Alienor Study's baseline eye examination. The design of this study has been approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in May 2006.

1958 British Birth Cohort

The 1958 British Birth Cohort originally consisted of 17,000 individuals, all born in one week in March 1958, and subsequently followed up at intervals with physical examination and collection of data on environmental, social and lifestyle factors (306). In 2002/3, when the participants were aged 44/45 years, a biomedical survey was undertaken of the 9377 individuals still actively participating in the study. Of all the subjects, 2502 were selected randomly and

underwent non-cycloplegic autorefraction, using the Retinomax 2 autorefractor (equipment costs precluded autorefraction of all subjects).

EPIC-Norfolk Eye Study

The European Prospective Investigation into Cancer (EPIC) study is a pan-European prospective cohort study designed to investigate the aetiology of major chronic diseases (307) EPIC-Norfolk , one of the UK arms of EPIC, recruited and examined 25,639 participants aged 40-79 years between 1993 and 1997 for the baseline examination (308). Recruitment was via general practices in the city of Norwich and the surrounding small towns and rural areas, and methods have been described in detail previously (309). Since virtually all residents in the UK are registered with a general practitioner through the National Health Service, general practice lists serve as population registers. Ophthalmic assessment formed part of the third health examination and this has been termed the EPIC-Norfolk Eye Study (310). In total, 8,623 participants were seen for the ophthalmic examination, between 2004 and 2011. Refractive error was measured using an autorefractor (Model 500, Humphrey Instruments, San Leandro, California, USA. The EPIC-Norfolk Eye Study was carried out following the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk & Waveney NHS Research Governance Committee (2005EC07L).

Erasmus Rucphen Family (ERF) Study

The ERF Study is a family-based cohort in a genetically isolated population in the southwest of the Netherlands with over 3,000 participants aged between 18 and 86 years. The rationale and study design of this study have been described elsewhere (311, 312). Cross-sectional examination took place between 2002 and 2005. Refractive error was measured using a Topcon RM-A2000 autorefractor (non-dilated). All measurements in these studies were conducted after the Medical Ethics Committee of the Erasmus University had approved the study protocols.

EUREYE Study

The EUREYE Study is multicentre, population-based cross-sectional study with retrospective and current exposure measurements primarily designed to study age-related macular degeneration. A detailed description of the study design has been reported (313). Briefly, 7 study centres, Bergen, Norway; Tallinn, Estonia; Belfast, Northern Ireland, United Kingdom; Paris-Creteil, France; Verona, Italy; Thessaloniki, Greece; and Alicante, Spain, were chosen primarily to maximize the range of latitude and lifestyle behaviours, including diet. Refractive data was not collected from the Spanish centre. The EUREYE Study aimed to enrol 800 to 900 persons 65 years and older in each of the 7 centres. The sample size calculations estimated that 6000 people would be required to detect a prevalence of AMD of mean ± SD 2% ± 0.5% at 95% confidence and a design effect of 2, to allow for the cluster (ie, country) effects. The sampling frame consisted of all persons 65 years or older who were included in the National Population Registry (Estonia), Patient Register (Northern Ireland, which includes all people registered with family physicians—around 98% of the local population), National Office for Statistics (Spain), and Municipal Register (France, Greece, Italy, and Norway) at the time the sample was requested. In each centre, the random sample was drawn by the statistical officers at the registries. Ethics approval was obtained at each centre from the relevant ethics committee. Study participants gave informed written consent prior to participation. Any participant who was unable to achieve a 0.3 logMAR (Snellen 20/40) in either eye underwent automatic or manual retinoscopy followed by refraction and recording of best-corrected acuity.

Gutenberg Health Study

The Gutenberg Health Study (GHS) is an on-going, population-based, prospective, observational cohort study in the Rhine-Main Region in Midwestern Germany with a total of 15,010 participants (314). The study sample was recruited from subjects aged between 35 and 74 years at the time of the examination. Exclusion criteria were insufficient knowledge of the German language to understand explanations and instructions, and physical or

psychic inability to participate in the examinations in the study centre. Refractive error was measured non-dilated using a Humphrey® Automated Refractor/Keratometer (HARK) 599 (Carl Zeiss Meditec, Jena, Germany). The study was approved by the Medical Ethics Committee of the University Medical Center Mainz and by the local and federal data safety commissioners.

KORA Study

KORA ("Kooperative Gesundheitsforschung in der Region Augsburg" which translates as “Cooperative Health Research in the Region of Augsburg”) is a population-based study of adults randomly selected from 430,000 inhabitants living in Augsburg and 16 surrounding counties in Germany (315). The collection was done in 4 separate groups from 1984-2001 (S1-S4). All survey participants are residents of German nationality identified through the registration office. In the KORA S3 and S4 studies 4,856 and 4,261 subjects have been examined implying response rates of 75% and 67%, respectively. 3,006 subjects participated in a 10-year follow-up examination of S3 in 2004/05 (KORA F3), and 3080 of S4 in 2006/2008 (KORA F4). The age range of the participants was 25 to 74 years at recruitment. The study was approved by the local ethics committee. Written informed consent was obtained from all participants before enrolment in accordance with the Declaration of Helsinki. For each subject, eyeglass prescriptions were measured in addition to an evaluation using the Nikon Retinomax. Refractive error was analysed, taking the mean measured spherical equivalent (SE) across both eyes (or SE in a single eye when both eyes were not measured) as the trait of interest.

The Montrachet 3C Study

Subjects of the Montrachet (Maculopathy Optic Nerve nuTRition neurovAsCular and HEarT diseases) study were recruited from an on-going population-based study, the Three-City (3C) study, on the vascular risk factors for dementia (305). The 3C-Study was designed to examine the relationship between vascular diseases and dementia in a community housing 9,294 persons aged 65 years and over. The participants were selected from the electoral rolls and were

only urban since they lived in 3 French cities, Bordeaux, Dijon and Montpellier. The 3C-Study began in 1999 and participants were evaluated every two years. A subgroup underwent ocular assessment in Bordeaux (Alienor study; see above (304)) and Dijon (Montrachet study).

In Dijon 4,934 subjects participated to the first run of the 3C-Study in 1999. They were followed every 2 years and at the fourth run undertaken in 2006/2007 they were still 3,137. Among them, 1,604 (51.1%) underwent an MRI at baseline and at the fourth year. We decided to include preferentially the participants having had an MRI and to complete the recruitment with participants without MRI. Therefore from October 22th, 2009 until March 31th, 2013, 913 volunteers with an MRI were recruited in the Montrachet study and 236 without and MRI. After approval by the regional ethics committee, the study was registered as 2009-A00448-49. Refractive error was determined using an autorefractor without cycloplegia (Tonoref II, Nidek, Aichi, Japan).

The POLA Study

The Pathologies Oculaires Liées à l'Age (POLA) Study is a population-based study aimed at identifying the risk factors of age-related eye diseases. The methods of this study have been published elsewhere (316). For inclusion in the study, participants needed to be a resident of Sète (South of France) and aged 60 years and over. According to the 1990 population census, there were almost 12,000 eligible residents, of whom our objective was to recruit 3,000. The population was informed of the study through the local media. We also contacted 4,543 residents individually by mail and telephone, using the electoral roll. The baseline examinations took place in a mobile unit equipped with ophthalmologic devices. Between June 1995 and July 1997, 2,584 participants were recruited. The study was approved by the ethics committee of the University Hospital of Montpellier, France.

Refractive error was measure using a Topcon RM-A7000 autorefractor, and refined subjectively when assessing best-corrected visual acuity. Cataract surgery was ascertained by the absence of the natural lens at slit lamp.

The Rotterdam Study I/II/III

The Rotterdam Study is a population-based study established in Rotterdam, the Netherlands (317). It consists of three cohorts. The original cohort, RS-I, started in 1990 and includes 7,983 subjects aged 55 years and older. The second cohort, RS-II, was added in 2000 and includes 3,011 subjects aged 55 years and older. The last cohort, RS-III, includes 3,932 subjects of 45 years of age and older and started in 2006. Refractive error was measured using a Topcon RM-A2000 autorefractor (non-dilated). Other ophthalmic baseline and follow-up examinations, which are still on-going, were described previously (318). The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports.

Thessaloniki Eye Study

The Thessaloniki Eye Study (TES) is a cross-sectional, population-based, epidemiologic study of chronic eye diseases in the Greek population of Thessaloniki. According to the National Statistical Service of Greece, Thessaloniki which is a major urban centre in Northern Greece is considered representative of the general population in the country. The initial recruitment frame of the TES consisted of 5,000 people, 60 years of age or older, who were identified randomly in February 1999 from approximately 321,000 persons registered in the municipality registers of the city of Thessaloniki. Subject recruitment is described in detail elsewhere (319). In summary, randomization was provided by the municipality statistical service. From the initial recruitment sample of the 5,000 names, 3,617 subjects were eligible and finally 2,554 participated in the study (participation rate 71%) (320). Study examination and data collection ended in March 2005. The study was approved by the Aristotle University Hospital Ethics Committee and the University of California Los Angeles Human Subject Protection Committee. If visual acuity was less than 20/30 with habitual correction, a full refraction was performed, and best-corrected visual acuity was measured.

Tromso Eye Study

The Tromsø Eye Study is a part of the Tromsø Study, a large comprehensive longitudinal population-based study started in 1974, and TES will take advantage of data collected in the present and previous Tromsø surveys. The Tromsø Study and the cohort profile have been described elsewhere (321). A total of 40 051 subjects have participated in at least one of the six surveys. The eye examinations were performed at the second visit. The study sample for the present study is based upon the official population registry and all subjects were residents of the municipality of Tromsø. A total of 19 762 subjects were invited to the first visit. Subjects invited to the first visit of the sixth Tromsø Study survey were all Tromsø residents aged 40–42 or 60–87 years (*n* = 12 578), a 10% random sample of individuals aged 30–39 years (*n* = 1056), a 40% random sample of individuals aged 43–59 years (*n* = 5787) and subjects who had attended the second visit of the fourth survey, if not already included in the three groups above (*n* = 341). A total of 12 984 subjects (65.7%) participated. Refraction was measured by Nidek AR 660A autorefractor (Nidek Co., Ltd. Gamagori, Japan). Spherical equivalent was calculated as spherical power plus half the cylindrical power in dioptres (D) and presented as the mean value of left and right eye (322).

TwinsUK

The TwinsUK adult twin registry, based at St. Thomas' Hospital in London, compromises over 12,000 predominantly female Caucasian ancestry twins, from throughout the United Kingdom (323). Twins largely volunteered unaware of the eye studies at the time of enrolment and gave fully informed consent under a protocol reviewed by the St. Thomas' Hospital Local Research Ethics Committee (EC04/015), which was performed in accordance with the Helsinki Declaration. Various eye phenotypes have been collected on a subset of twins. Refractive error was measured using non-cycloplegic autorefraction (ARM-10 autorefractor, Takagi Seiko, Japan).

8.5 Supplementary Information for the cohorts contributing to the presented Consortium for Refractive Error and Myopia (CREAM) analysis

Recruitment of participants and phenotypic assessment

ALSPAC. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Participant recruitment has been described previously [5]. Details of the phenotypes available and data access can be found at: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/. Pregnant women with an expected date of delivery between 1st April 1991 and 31st December 1992, resident in the former Avon health authority area in Southwest England, were eligible to participate in this birth cohort study. 13,761 women were recruited. Data collection has been via various methods including self-completion questionnaires sent to the mother, to her partner and after age 5 to the child; direct assessments and interviews in a research clinic. Non-cycloplegic autorefraction (Canon R50 instrument) was performed when participants attended a research clinic visit, at the target ages of approximately 7, 10, 11, 12 and 15 years. DNA samples were available for 11,343 ALSPAC Children, prepared from either blood samples or lymphoblastoid-transformed cell lines. Mothers completed a questionnaire when the children were aged, on average, 8.6 years old. A child was classified as spending a "high" amount of time performing nearwork if their mother reported they spent either "1–2 hours" or "3 or more hours", and as spending a "low" amount of time on nearwork otherwise, in response to the question, "On normal days in school holidays, how much time on average does your child spend each day reading books for pleasure?". The item, "On a weekend day, how much time on average does your child spend each day out of doors in summer?" was used to classify children as spending a "high" amount of time outdoors if the response was "3 or more hours" and as "low" otherwise.

BATS

The Brisbane Adolescent Twin Study is an ongoing study of adolescent and young-adult monozygotic (MZ) and dizygotic (DZ) twin pairs (2720 individuals) and their siblings (1179)[6]. Twins were initially recruited to the study from primary and secondary schools in South East Queensland in 1992, with new twins added at various intervals. In addition, a small number of twins have been recruited through word of mouth, or through the Australian Twin Registry. The study was approved by the human research ethics committee at the QIMR Berghofer Medical Research Institute. Twins have undergone a variety of phenotypic assessments. A 40-ml blood sample is collected from participants and parents at the initial assessment. A subset of participants also completed an extensive eye examination as part of

the Twins Eye Study in Tasmania. Autorefraction was performed using Humphrey-598 Automatic Refractor / Keratometer (Carl Zeiss Meditec, Inc., Miami, Florida, USA).

GZT

The Guangzhou Twin Eye Study was launched in 2006, and it has completed eight consecutive annual follow-up examinations, with more than 1200 twin pairs participating. In brief, twins born in Guangzhou aged 7 to 15 years received annual eye examinations, including cycloplegic refraction, from 2006 onwards. Those with manifest strabismus, amblyopia, nystagmus, post-refractive surgery, or any ocular disease causing best-corrected visual acuity less than 20/25 were excluded from the current analysis. The study was conducted in accordance with the tenets of the World Medical Association's Declaration of Helsinki and was approved by the Ethics Review Board of the Zhongshan Ophthalmic Center of Sun Yat-Sen University. Written informed consent was obtained from the parents or legal guardians of the participants. Cycloplegia was induced with 2 drops of 1% cyclopentolate, administered 5 minutes apart, with a third drop administered after 20 minutes. Cycloplegia and pupil dilation were evaluated after an additional 15 minutes. Cycloplegia was considered complete if the pupil dilated to 6 mm or greater and a light reflex was absent. If not, another 20 minutes observation was taken, and refractive measurement was taken regardless of the presence or absence of light reflex. Refraction was performed with an auto-refractor (Topcon KR-8800, Tokyo, Japan) after cycloplegia. The questionnaire used in the study was designed by a World Health Organization (WHO) working group. It included the questions on indoor and outdoor activities for weekdays and weekend days separately. In each section, daily activity was divided into four types: nearwork activity (including reading, writing, drawing), middle-distance activity (including watching television or movies and playing video games), indoor leisure activity (including singing, housework, dancing in doors), and outdoor activity (including sports, walking outside). Participants were asked to report daily time for each of the activities into 3 categories - not at all, less than one hour or more than one hour. If "more than one hour" was reported, exact time was further specified. During school terms (February to July, September to December) the average time for each type activity was calculated as (5×weekday + 2×weekend)/7. During holidays, the daily visual activity refers to weekend information. In China, every year has 9 months semester days and 3 months summer/winter holidays. The average nearwork and outdoor activity per day in the past year was calculated as (9×semester day time + 3×holidaytime)/12.

RAINE

The Western Australian Birth Cohort (Raine) Study [7] is one of the largest ongoing prospective cohort studies. It was established in 1989 by recruiting 2900 pregnant women at 16-18 weeks of gestation in Perth. The original aim of the study was to investigate how events during pregnancy and at birth influence the health and wellbeing of the newborns. This cohort has gone on to be examined every 2 years by different research groups. At the 20 year follow-up of the Raine Cohort were invited to participate in the Raine Eye Health Study (REHS) and undertake a comprehensive eye examination. This study was approved by the Human Research Ethics Committee at the University of Western Australia. During eye examination, post-cycloplegic autorefraction was performed in 1344 participant using the Nidek ARK-510A (NIDEK Co.Ltd, Japan) autorefractor. As part of the study questionnaire, individuals were asked to report their time spent outdoors and had four possible responses to the question "In the summer, when not working at your job or at school, what part of the day do you spend outside?": none, < ¼ of the day, approximately half of the day and > ¾ of the day. "None" and "<¼ of the day" groups were combined due to low numbers in the "none" category. DNA samples and consents for 1494 participants were available from the previous assessments of the cohort. Individuals with refractive surgery or corneal eye diseases were excluded from the analysis.

SCORM

A total of 1,979 children in grades 1, 2, and 3 from three schools were recruited from 1999 to 2001 with detailed information described elsewhere [8]. The children were examined on the school premises annually by a team of eye care professionals. The GWAS was conducted in a subset of Chinese children of 1,116 participants [9]. The phenotype used in the cross-sectional study was based on the SE measured on the 4th annual examination of the study (children at age 10 to 12 years). Complete post-filtering data on measurements and SNP data were available in 994 SCORM children. Parents were asked through questionnaire to quantify nearwork activity (reading, writing, computer use, playing video games) in hours per day per activity on weekdays and weekends. The average number of outdoor activity hours per day was calculated using the formula: (hours spent on weekday) x 5 + (hours spent on weekend day) x 2)/7. The total outdoor activity was defined as the sum of outdoor leisure and outdoor sporting activities [10].

STARS

STARS is a population-based survey of Chinese families with children residing in the south-western and western region of Singapore. Disproportionate random sampling by 6-month age groups resulted in the recruitment and subsequent eye examination of 3,009 Chinese children between May 2006 and November 2008. Details of the study design and methodology have been previously described [11]. A total of 1,451 samples from 440 nuclear families underwent eye examinations and were included for genome-wide genotyping. In all, 407 children with SE measurement had complete post-filtered genotype data. Near work activities were recorded in number of hours per day. Activities included reading, colouring and drawing, watching television, playing television games, playing hand-held video games and using computers [12]. The outdoor activity questionnaire was similar to that used for SCORM [10].

TEDS

In the initial Twins Early Development Study (TEDS) over 15,000 families of twins born in England and Wales in 1994, 1995 and 1996 were recruited, and the sample remains representative of the UK population [13]. Ethical approval for TEDS and TEDS myopia study has been provided by the Institute of Psychiatry ethics committee, reference number 05/Q0706/228 and PNM/11/12-140 respectively. A subset of 2625 families were selected for the TEDS Myopia study. This sample was selected to include families from TEDS cohort 2 where twins had returned the web questionnaire that included eyesight questions and additional families were added from other cohorts if twins had GWAS data. We excluded from the analyses children with severe current medical problems and families who were not contactable or who lived overseas. Postal questionnaires were sent to a subset of 2,625 families (parents and twins) inviting participation in the myopia study and consent was requested from the parents as well from the twins to contact their optician for a recent refraction. Study questionnaires were sent to the optometrist of 2,283 consenting twins; non-cycloplegic subjective refraction measurements were obtained for 1,996 individuals. DNA samples were available for 3,152 TEDS participants. Multiple child and parent questionnaires, in addition to teacher questionnaires, web-based testing and assessments at home, have been conducted over the twins' life-course; at the age of fourteen a questionnaire was sent to the twins where they asked how much time they spent on a number of extra-curricular activities. The number of hours per week spent on a number of activities, including computer games, reading for fun and hanging outside with friends, was requested.

TEST

Commencing in the late 2000, 1372 participants were recruited to the Twins Eye Study Tasmania through various methods including piggy-backing existing studies where twins had been recruited, utilizing the national twin registry, word-of-mouth and local media publicity and directly approaching schools [14]. Ethical approval was obtained from the Royal Victorian Eye and Ear Hospital, the University of Tasmania, the Australian Twin Registry (ATR). As part of the eye examination, post-cycloplegic autorefraction was completed in all participants using Humphrey-598 Automatic Refractor / Keratometer (Carl Zeiss Meditec, Inc., Miami, Florida, USA). In children, buccal swabs or Oragene saliva samples were collected. In adolescents, or when repeat examination was conducted several years later, a blood sample was taken and those participants who were now adults signed their own consent.

WESDR

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) is a population-based observational cohort study of diabetic patients from an eleven-county area in southern Wisconsin since 1979. Participants have gone through an initial and 6 follow-up examinations performed in a van near their residences. Each examination had an extensive ophthalmologic component including measurement of subjective refraction and best corrected visual acuity. For the current analysis, subjective refraction measured at the first visit in adult patients with Type 1 diabetes was used. Further details about recruitment and ophthalmologic exam could be found elsewhere [15].

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